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Mathematical simulation of gas transport and acid/base regulation by blood flowing in microvessels

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Rice University, 1991
RICE UNIVERSITY

MATHEMATICAL SIMULATION OF GAS TRANSPORT AND ACID/BASE REGULATION BY BLOOD FLOWING IN MICROVESSELS

by

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A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

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ABSTRACT

MATHEMATICAL SIMULATION OF GAS TRANSPORT AND ACID/BASE REGULATION BY BLOOD FLOWING IN MICROVESSELS

by

Nancy Shu-Hui Huang

A simple model has been developed for simulation of oxygen (O₂) transport by red blood cells (RBCs) to and from blood flowing in vessels with diameters of 20 microns and larger where a substantial fraction of the microcirculatory O₂ transport occurs. This model is derived from a more complete model which has been validated experimentally. Detailed calculations of the oxygen concentration distribution reveal that the dominant resistance to O₂ transport is distributed in the plasma and that relatively little resistance is present within or in the immediate vicinity of the RBCs. Based on these findings, the complete model is simplified from four simultaneous nonlinear partial differential equations (PDEs) to one PDE by: (1) assuming chemical equilibrium within the RBCs, (2) neglecting intracellular and extracellular boundary layer resistances, and (3) incorporating transport in the RBC-free plasma region adjacent to the vessel wall into the boundary condition. The simplified model is much easier to apply mathematically to new situations. A comparison between the two models shows that they give similar predictions which agree well with experimental measurements.

In order to investigate the coupling between the oxygen and carbon dioxide (CO₂) transport, an extended model is developed to incorporate CO₂, as well as the
various blood buffer systems that are closely connected to the transport of these gases. The blood is treated as two continuous coexisting phases: a RBC phase and a plasma phase. The microvessel is divided into two regions: the central, RBC-rich and the outer, cell-free region. The radial distribution of RBCs, and transport of various species due to bulk convection are taken into account. Chemical and transport processes which are included in the model are (1) interactions of hemoglobin with \( O_2 \) and \( CO_2 \), (2) the Bohr and Haldane effects, (3) \( CO_2 \) hydration/dehydration reactions, (4) buffering actions of hemoglobin and plasma proteins, and (5) anion exchange across the RBC membrane. Predictions of the discrete model of simultaneous \( O_2/CO_2 \) transport by flowing blood are shown to be in excellent agreement with prior workers' experimental results from large artificial membrane tubes. A previous mathematical model which treats blood as a homogeneous continuum and uses a local chemical equilibrium approximation to describe the gas transport is shown to satisfactorily predict the amount of \( O_2 \) transport for blood oxygenation accompanied by \( CO_2 \) elimination cases, but significantly underpredict \( O_2 \) transfer for blood deoxygenation accompanied by \( CO_2 \) uptake cases. Furthermore, this previous model disagrees substantially with the \( CO_2 \) transport results under both oxygenation and deoxygenation conditions.
To my parents Yueh-Shing and Tong-Thy Huang
ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following persons for their help and support in the completion of this research project and in the writing of this thesis:
Dr. J. David Hellums for serving as my thesis advisor, for his insight and guidance in directing this research, for his support and encouragement, and for many helpful discussions and suggestions.
Dr. Larry V. McIntire for serving as a member of my thesis committee, and for his helpful professional advices.
Dr. John S. Olson for serving as a member of my thesis committee, and for many stimulating discussions.
Dr. Colin B. Mcclay for his constructive discussions on the experimental aspect of this investigation.
Dr. Douglas D. Lemon for many helpful discussions, valuable suggestions, and use of his experimental data.
The faculty of the Chemical Engineering Department for contributing to my education and intellectual development.
All the special friends in the Institute of Biosciences and Bioengineering and the Department of Chemical Engineering for their friendship and moral support.
Ms. Marcella Estrella and Ms. Nancy Turner for their technical assistance.
My parents, Yueh-Shing and Tong-Thy Huang, for many years of sacrifice and encouragement.

This project was supported financially by the Texas Advanced Technology Program Grant 4073, the Robert A. Welch Foundation Grant C-612, and NIH Grants GM 35649 and HL 19824.
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NOMENCLATURE

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<tr>
<td>(a)</td>
<td>particle radius of red cell</td>
</tr>
<tr>
<td>(a_i)</td>
<td>(i=\text{integer, equilibrium constant in equations for the ODC models (Table 2.1)} ) under standard conditions ((T=37 , ^\circ C, , pH=7.4, , P_{CO2}=40 , \text{mmHg and the molar ratio of } [DPG]/[Hb_4]=0.9 ) where Hb_4 denotes hemoglobin)</td>
</tr>
<tr>
<td>(A)</td>
<td>constant in the velocity profiles which needs to be determined</td>
</tr>
<tr>
<td>(\dot{A})</td>
<td>carbonic anhydrase activity</td>
</tr>
<tr>
<td>(\dot{A}_{pl})</td>
<td>carbonic anhydrase activity in the plasma</td>
</tr>
<tr>
<td>(\dot{A}_{rbc})</td>
<td>carbonic anhydrase activity in the RBC</td>
</tr>
<tr>
<td>(B)</td>
<td>blunting factor in the velocities profiles</td>
</tr>
<tr>
<td>(B_i)</td>
<td>(i=\text{integer, equilibrium constant in equations for ODC models (Equations (2.2) or (2.4)) under nonstandard conditions)}</td>
</tr>
<tr>
<td>(B_\sigma)</td>
<td>Bromley interaction term</td>
</tr>
<tr>
<td>(C_{DH})</td>
<td>Debye-Hückel constant</td>
</tr>
<tr>
<td>(C_{heme})</td>
<td>total heme concentration in the suspension (continuum approach)</td>
</tr>
<tr>
<td>(C_{heme,rbc})</td>
<td>total heme concentration inside the RBC</td>
</tr>
<tr>
<td>(d)</td>
<td>half-thickness of the artificial membrane film</td>
</tr>
<tr>
<td>(D)</td>
<td>constant in the velocity profiles which needs to be determined</td>
</tr>
<tr>
<td>(D_i)</td>
<td>effective diffusion coefficient of species (i) in the suspension (continuum approach)</td>
</tr>
<tr>
<td>(D_i^0)</td>
<td>diffusion coefficient of ionic species (i) in infinitely dilute solution</td>
</tr>
<tr>
<td>(D_{i,34%Hb})</td>
<td>diffusion coefficient of species (i) in concentrated hemoglobin solution</td>
</tr>
<tr>
<td>(D_{i,H2O})</td>
<td>diffusion coefficient of species (i) in water</td>
</tr>
<tr>
<td>(D_{i,k})</td>
<td>diffusion coefficient of species (i) in phase (k)</td>
</tr>
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</table>
\(D_{i,pt}\) diffusion coefficient of species \(i\) in the plasma

\(D_{i,T_0}\) diffusion coefficient of species \(i\) at temperature \(T_0\)

\(D_{O2,eff}\) effective diffusivity of \(O_2\) under shared condition

\(D_{O2,pl}\) shear-induced \(O_2\) diffusivity in the plasma phase

\(D_{O2,rbc}\) shear-induced \(O_2\) diffusivity in the red cell phase

\(D_{O2,UF}\) effective diffusivity of \(O_2\) under unshared condition

\(D_{rbc}\) shear-induced diffusion coefficient of RBCs

\(\tilde{D}_i\) diffusion coefficient for ionic species \(i\) in the suspension

\(\tilde{D}_{i,pl}\) diffusion coefficient of ionic species \(i\) in the plasma

\(E_c\) equilibrium parameter in the Easton ODC model (Table 2.1)

\(E_i\) intercept of linearized dissolved \(CO_2\) content vs. total \(CO_2\) content curve

\(E_{lp}\) lumped parameter in the Easton ODC model

\(f_{water}\) volume fraction of water inside the RBC

\(F\) Faraday constant

\(F_s\) slope of linearized dissolved \(CO_2\) content vs. total \(CO_2\) content curve

\(Flux_{CO2}\) \(CO_2\) flux crossing the RBC wall, a function of \(r\) and \(z\)

\(Flux_{HCO3}\) net flux of extracellular \(HCO_3^-\) entering the RBCs or, equivalently, the net flux of intracellular \(Cl^-\) entering the plasma

\(Flux_{O2}\) \(O_2\) flux crossing the RBC wall, a function of \(r\) and \(z\)

\(g\) a measure of the departure of the hemoglobin-oxygen binding reaction from equilibrium \((0 \leq g \leq 1)\); in the limit of reaction equilibrium, \(g=1\)

\(G_i\) intercept of linearized dissolved \(CO_2\) content vs. \(HCO_3^-\) content curve

\(GF\) geometric factor; \(GF=r_d/r_c\) in both Diller et al.'s and Schmukler and Chien's systems, and \(GF=4d/\pi r_c\) in Boland et al.'s system

\(h(r)\) hematocrit profile, the volume fraction of RBCs at any radius \(r\)

\(h_m\) constant in the hematocrit profile which needs to be determined
$H_D$  discharge hematocrit
$H_T$  tube hematocrit
$H_s$  slope of linearized dissolved CO$_2$ content vs. HCO$_3^-$ content curve
$I$  ionic strength of the medium calculated using molar concentrations
$I_m$  ionic strength of the medium calculated using the molality of ions
$j$  dissociation rate constants for O$_2$ binding to hemoglobin
$j'$  association rate constants for O$_2$ binding to hemoglobin
$k$  dissociation rate constant of Reaction (6.V)
$k'$  association rate constant of Reaction (6.V)
$k_a$  deoxyhemoglobin-CO$_2$ reaction forward rate constant (Reaction (6.VI))
$k'_a$  oxyhemoglobin-CO$_2$ reaction forward rate constant (Reaction (6.VII))
$k_d$  deoxyhemoglobin-CO$_2$ reaction reverse rate constant (Reaction (6.VI))
$k'_d$  oxyhemoglobin-CO$_2$ reaction reverse rate constant (Reaction (6.VII))
$k_i$  $i=1, 3, 4$ and $6$; association rate constants for anion-protein complex
$k_{i_1}$  $i=2$ and $5$; outward translocation rate constant of bound anion
$k_{i}$  $i=1, 3, 4, 6$; dissociation rate constants for anion-protein complex
$k_{i_1}$  $i=2$ and $5$; inward translocation rate constant of bound anion
$k_{\text{trans}}$  translocation rate constant of bound Cl$^-$ and HCO$_3^-$ in the simplified ping-pong model
$k_u$  CO$_2$ hydration rate constant
$k_v$  H$_2$CO$_3$ dehydration rate constant
$K'$  apparent first acid dissociation constant for H$_2$CO$_3$
$K_1$  first acid dissociation constant for H$_2$CO$_3$
$K_2$  second acid dissociation constant for H$_2$CO$_3$
$K_a$  amino group ionization constant for deoxygenated hemoglobin carbamate

(Reaction (6.VI))
\(K_a\) amino group ionization constant for oxygenated hemoglobin carbamate (Reaction (6.VII))

\(K_A\) equilibrium association rate constant for Cl\(^-\)-protein and HCO\(_3\)^-protein complexes in the simplified ping-pong model

\(K_{buffer}\) equilibrium constant of the buffer

\(K_{CO2}\) CO\(_2\) permeability of the artificial membrane

\(K_{DPG}\) pH-dependent association constant of DPG and hemoglobin

\(K_{eq}\) pseudo equilibrium constant in approximate CO\(_2\) dissociation curve

\(K_i\) \(i=1\) and \(3\); microscopic equilibrium association constants for Cl\(^-\)-protein complex in the inward and outward membrane compartments, respectively (Equation 5.10)

\(K_i\) \(i=4\) and \(6\); microscopic equilibrium association constants for HCO\(_3\)^-protein complex in the outward and inward membrane compartments, respectively (Equation 5.10)

\(K_{1,Cl}\) dissociation constant of the carrier-Cl\(^-\) complex

\(K_{1,HC03}\) dissociation constant of the carrier-HCO\(_3\)^- complex

\(K_M\) Michaelis constant

\(K_{O2}\) O\(_2\) permeability of the artificial membrane

\(K_{water}\) dissociation constant of water

\(K_z\) amino group ionization constant for the reduced hemoglobin, (Reaction (6.IV))

\(L\) axial length of the artificial membrane tube

\(m\) O\(_2\) carriage potential of hemoglobin calculated based on the local operating slope of the ODC (Equation (2.19b))

\(m'\) constant in the hematocrit profile which needs to be determined

\(m_1\) local operating slope along the unsaturated portions of the ODC
\( m_2 \)  
local operating slope along the almost saturated portions of the ODC

\( M \)  
\( O_2 \) carriage potential of hemoglobin calculated based on the overall operating slope of the ODC (Equation (2.20b))

\( n \)  
Hill equilibrium parameter, an index for cooperativity of hemoglobin

\( N_i \)  
flux of species i

\( pH \)  
\( - \log_{10} \) [hydrogen ion concentration]

\( pH_{pl} \)  
extracellular \( pH \)

\( pH_{pl,in} \)  
extracellular \( pH \) of inlet RBC suspension

\( pH_{rbc} \)  
intracellular \( pH \)

\( pH_{rbc,in} \)  
intracellular \( pH \) of inlet RBC suspension

\( pK \)  
\( - \log_{10} \) [equilibrium constant]

\( P_{50} \)  
Hill equilibrium parameter, \( P_{O_2} \) at which \( O_2 \) saturation is 50%

\( P_{CO_2} \)  
partial pressure of \( CO_2 \)

\( P_{CO_2,ext} \)  
partial pressure of \( CO_2 \) in the gas space surrounding the artificial membrane tube

\( P_{CO_2,in} \)  
inlet partial pressure of \( CO_2 \)

\( P_{Cl} \)  
phenomenological permeability coefficient of \( Cl^- \) in the constant field electrodiffusion model (Equation (5.1))

\( P_{HCO_3} \)  
phenomenological permeability coefficient of \( HCO_3^- \) in the constant field electrodiffusion model (Equation (5.1))

\( P_{O_2} \)  
partial pressure of \( O_2 \)

\( P_{O_2}^* \)  
\( P_{O_2} \) at which maximum slope for modified Easton model occurs

\( \overline{P_{O_2}} \)  
equilibrated mixed mean oxygen tension in the collected sample

\( P_{O_2,ext} \)  
partial pressure of \( O_2 \) in the gas space surrounding the artificial membrane tube

\( P_{O_2,in} \)  
inlet partial pressure of \( O_2 \)
$P_{O2,NS}$  partial pressure of $O_2$ under nonstandard conditions (Equation (2.3))

$P_{O2,S}$  partial pressure of $O_2$ under standard conditions (Equation (2.3))

$Q$  volumetric flow rate of the RBC suspension in the tube

$r$  radial coordinate

$r'$  dimensionless concentration of $HCO_3^-$ in the RBC-rich plasma region

$r''$  dimensionless concentration of $HCO_3^-$ in the RBC-free plasma region

$r_c$  inner radius of the artificial membrane tube

$r_o$  outer radius of the artificial membrane tube

$r_r$  radius of the cell-rich region, the difference of tube radius and the thickness of the cell-free layer

$r_{rbc}$  radius of the RBC

$R$  gas constant

$R_{GD}$  Gibbs-Donnan ratio

$R_{HbCO2}$  rate of formation of hemoglobin carbamate per unit RBC volume

$R_{HbO2}$  rate of formation of oxyhemoglobin per unit RBC volume

$R_{HCO3,pl}$  rate of consumption of $CO_2$ per unit plasma volume by the hydration reaction

$R_{HCO3,rbc}$  rate of consumption of $CO_2$ per unit RBC volume by the hydration reaction catalyzed by the intracellular carbonic anhydrase

$R_{i,k}$  rate of reaction which generates $i$ in phase $k$

$R_{O2HbCO2}$  rate of formation of oxyhemoglobin carbamate per unit RBC volume

$s$  red cell surface area

$s'$  dimensionless concentration of $Cl^-$ in the RBC-rich plasma region

$(s^s)_{rbc}$  surface to volume ratio of the RBC

$s_{lp}$  slip constant in the RBC velocity profile

$S$  fractions of available hemoglobin sites saturated with $O_2$
\( \bar{S} \) physically mixed mean oxyhemoglobin saturation in the equilibrated sample

\( S_{\text{max}} \) scaling factor of the modified Easton model (Table 2.1)

\( S_{\text{min}} \) scaling factor of the modified Easton model (Table 2.1)

\( S_{\text{mm}} \) calculated overall mixed mean oxyhemoglobin saturation in the tube

\( S_{\text{P02=P02,ext}} \) fractional saturation of the hemoglobin molecules under the O\(_2\) partial pressure of \( P_{O2,\text{ext}} \)

\( S_{\text{P02=P02,in}} \) fractional saturation of the hemoglobin molecules under the O\(_2\) partial pressure of \( P_{O2,\text{in}} \)

\( S_{\text{sa}} \) space average oxygen saturation of the hemoglobin molecules

\( t \) dimensionless axial coordinate

\( t_{\text{rbc}} \) maximum thickness of the RBC

\( T \) temperature of the system

\( T_o \) reference temperature

\( T_{\text{p1}} \) unloaded form of the transporter in which a single anion binding/transport site is accessible to only extracellular anions

\( T_{\text{rbc}} \) unloaded form of the transporter in which a single anion binding/transport site is accessible to only intracellular anions

\( T_{\text{tot}} \) total number of anion transporters per RBC

\( TX_{\text{p1}} \) complex of the transporter with extracellular anion X (Cl\(^-\))

\( TX_{\text{rbc}} \) complex of the transporter with intracellular anion X (Cl\(^-\))

\( TY_{\text{p1}} \) complex of the transporter with extracellular anion Y (HCO\(_3^-\))

\( TY_{\text{rbc}} \) complex of the transporter with intracellular anion Y (HCO\(_3^-\))

\( u \) dimensionless concentration of O\(_2\) inside the RBC

\( v \) dimensionless concentration of CO\(_2\) inside the RBC

\( v'' \) dimensionless concentration of CO\(_2\) in the RBC-free plasma region
\( v_{max} \)  
the maximum reaction rate of an enzyme-catalyzed reaction described via Michaelis-Menten equation

\( v_0 \)  
initial rate of an enzyme-catalyzed reaction described via Michaelis-Menten equation

\( V(r) \)  
velocity profile in the \( z \) direction

\( \vec{V} \)  
velocity vector

\( \langle V \rangle \)  
average velocity in the tube; \( \langle V \rangle = \frac{Q}{\pi r_c^2} \)

\( V_{pl}(r) \)  
plasma velocity at radius \( r \) in the cell-rich region

\( V_{′pl}(r) \)  
plasma velocity at radius \( r \) in the cell-free region

\( \langle V \rangle_{pl} \)  
average plasma velocity; \( \langle V \rangle_{pl} = \frac{Q(1-H_D)}{\pi r_c^2 (1-H_T)} \)

\( V_{rbc}(r) \)  
RBC velocity at radius \( r \) in the cell-rich region

\( \langle V \rangle_{rbc} \)  
average red cell velocity; \( \langle V \rangle_{rbc} = \frac{Q \ H_D}{\pi r_c^2 \ H_T} \)

\( V_R \)  
volume of the CSTR reactor

\( w \)  
dimensionless concentration of \( H^+ \) inside the RBC

\( w' \)  
dimensionless concentration of \( H^+ \) in the RBC-rich plasma region

\( w'' \)  
dimensionless concentration of \( H^+ \) in the RBC-free plasma region

\( x \)  
dimensionless radial coordinate in the RBC-rich plasma region

\( x' \)  
dimensionless radial coordinate in the RBC-free plasma region

\( X_{pl} \)  
exttracellular \( Cl^- \) concentration

\( X_{rbc} \)  
intracellular \( Cl^- \) concentration

\( Y_{pl} \)  
exttracellular \( HCO_3^- \) concentration

\( Y_{rbc} \)  
intracellular \( HCO_3^- \) concentration

\( z \)  
axial coordinate

\( Z_i \)  
electrical valence of ion \( i \)

\([\text{buffer}]_{pl}\)  
molar concentration of the buffer in the extracellular medium

\([C_i]\)  
concentration of species \( i \) in the suspension (continuum approach)
\[ [C_i]_k \] concentration of species i in phase k

\[ [C_i]_{mm} \] mixed mean concentration of species i in the suspension (continuum approach)

\[ [C_i]_{mm,rbc} \] mixed mean concentration of species i inside the RBC

\[ [C_i]_{mm,pt} \] mixed mean concentration of species i in the plasma

\[ [C_i]_{mm,wb} \] mixed mean concentration of species i in the whole blood

\[ [C_i]_o \] normalized concentration for species i

\[ [C_i]_{pl} \] concentration of species i in the RBC-rich plasma region

\[ [C_i]^\prime_{pl} \] concentration of species i in the RBC-free plasma region

\[ [C_i]_{pl,calc} \] predicted concentration of species i in the extracellular phase

\[ [C_i]_{pl,exp} \] experimentally determined concentration of species i in the extracellular phase

\[ [C_i]_{pl,in} \] inlet concentration of species i in the extracellular phase

\[ [C_i]_{pl,out} \] outlet concentration of species i in the extracellular phase

\[ [C_i]_{pt,rw} \] concentration of species i at the RBC wall in the plasma

\[ [C_i]_{rbc} \] concentration of species i inside the RBC

\[ [C_i]_{rbc,calc} \] predicted concentration of species i in the intracellular phase

\[ [C_i]_{rbc,exp} \] experimentally determined concentration of species i in the intracellular phase

\[ [C_i]_{rbc,in} \] inlet concentration of species i in the intracellular phase

\[ [C_i]_{rbc,out} \] outlet concentration of species i in the intracellular phase

\[ [C_i]_{rbc,rw} \] concentration of species i at the RBC wall in the RBC

\[ [C_i]_{total} \] total concentration of species i

\[ [C_i]_{total,in} \] total inlet concentration of species i

\[ [C_i]_{total,out} \] total outlet concentration of species i

\[ [CO_2]_{reacted} \] concentration of reacted CO\(_2\) in blood (continuum approach)
$[CO_2]_{total}$ total CO$_2$ content which including HCO$_3^-$, carbamino hemoglobin, dissolved CO$_2$ and H$_2$CO$_3$

$[DPG]$ intracellular concentration of 2,3-diphosphoglycerate

$\bar{O}_2$ physically mixed mean oxygen concentration in the equilibrated sample

$[O_2]_{total}$ total O$_2$ content which including both oxyhemoglobin and dissolved O$_2$

$[S]_o$ initial substrate concentration of an enzyme-catalyzed reaction described via Michaelis-Menten equation

$\alpha_i$ effective solubility coefficient of species $i$ in the suspension (continuum approach)

$\alpha_{i,H_2O}$ solubility coefficient of species $i$ in water

$\alpha_{i,pl}$ solubility coefficient of species $i$ in plasma

$\alpha_{i,rbc}$ solubility coefficient of species $i$ in RBC

$\alpha_{i,saline}$ solubility coefficient of species $i$ in saline

$\beta$ amount of base added or acid removed per pH unit change which can be determined experimentally

$\beta_{pl}$ extracellular buffer capacity

$\beta_{rbc}$ intracellular buffer capacity

$\gamma$ local shear rate

$\Gamma$ temperature correction constant in Equation (6.50)

$\gamma_{avg}$ average shear rate

$\gamma_i$ activity coefficient of species $i$

$\delta$ thickness of the RBC-free layer

$\Delta[C_i]_{total}$ total content change of species $i$

$\zeta$ empirical parameter in the calculation of shear-induced diffusivity of RBCs (Equation (2.16))
\(\eta_{CO2}\) effective, lumped mass transfer coefficient for CO\(_2\) in Lemon's stopped-flow apparatus

\(\kappa\) equilibrium parameter in the equations for ODC models (Table 2.1) under standard conditions

\(\kappa_S\) equilibrium parameter in the modified Easton ODC model under standard conditions (Equation (2.7))

\(\kappa_{NS}\) equilibrium parameter in the modified Easton ODC model under nonstandard conditions (Equation (2.7))

\(\lambda_\alpha\) (or \(\lambda^\prime\)) pH-dependent association constant for CO\(_2\) binding for the \(\alpha\)-amino groups of the \(\alpha\)-chain of hemoglobin

\(\lambda_\beta\) (or \(\lambda^\prime\)') pH-dependent association constant for CO\(_2\) binding for the \(\alpha\)-amino groups of the \(\beta\)-chain of hemoglobin

\(\lambda_i\) equivalent conductance of ion \(i\) in solution

\(\lambda_i^o\) equivalent conductance of ion \(i\) in infinitely dilute solution

\(\mu\) viscosity of the solvent

\(\pi\) constant=3.14159...

\(\sigma\) empirical parameter in the calculation of shear-induced diffusivity of RBCs (Equation (2.16))

\(\sigma_1\) amount of H\(^+\) released per O\(_2\) molecule that is bound to hemoglobin (Reaction (6.IV))

\(\sigma_2\) amount of H\(^+\) released per CO\(_2\) molecule that is bound to deoxy-hemoglobin (Reactions (6.IV) and (6.VI))

\(\sigma_3\) amount of H\(^+\) released per CO\(_2\) molecule that is bound to oxyhemoglobin (Reactions (6.IV) and (6.VII))

\(\upsilon\) turnover rate of the anion transporter

\(\phi\) the moles of CO\(_2\) bound per mole hemoglobin tetramer
\( \psi \)  

electrical potential (Equation (5.1))

\( \Psi_m \)  

membrane potential (Equation (6.51))
CHAPTER 1

INTRODUCTION

Blood can be described as a circulating organ which ensures the optimal supply of oxygen ($O_2$) and essential nutrients and removal of carbon dioxide ($CO_2$) and other metabolic by-products from all the cells of the body. Blood is an intricate physicochemical system, even when one isolates the phenomena governing its role as a carrier of $O_2$ and $CO_2$. Basically the complexity arises from: (1) the division of the blood into effectively two phases, the erythrocytes (red blood cells, RBCs) and the plasma, between which there is transmembrane exchange of gases and anions; (2) the simultaneous interactions of RBC hemoglobin with $O_2$, $CO_2$, hydrogen ions ($H^+$s) and the metabolic intermediate, 2,3-diphosphoglycerate (DPG); and (3) intra- and extracellular hydration/dehydration reactions of $CO_2$ and buffering of $H^+$ by hemoglobin and plasma proteins. Consequently, blood $O_2$ and $CO_2$ chemistries are no more separable than they are from acid/base chemistry, and the coupling between the blood-gas transfer and acid/base balance is significant. Additionally, analysis of gas transport and $pH$ regulation by blood flowing in blood vessels or artificial, permeable membrane tubes requires information on the hydrodynamic characteristics of blood flowing through narrow tubes. Therefore, blood-gas transport is mediated not only by chemical reaction, but also by diffusion and convection. Thus, many of the related issues involved concern physicochemical phenomena and fluid mechanics.

One long-standing problem is to determine the relative $CO_2$ transport resistances within capillary blood and metabolizing tissue. This requires analysis and/or suitably scaled experimental modeling of mass transfer and chemical reactions in capillaries containing flowing RBCs. Several studies on the transport of $O_2$ in the microcirculation
indicated that the resistance to $O_2$ transport in blood is significant in comparison to the resistance in the surrounding tissue (Hellums, 1977; Artigue and Bruley, 1983; Honig et al., 1984; Federspiel and Popel, 1986). Hellums (1977) was the first to give a clear mathematical estimate of this effect; his analysis showed that the fraction of total $O_2$ transport resistance that resides inside the capillary is influenced significantly by the discrete nature of blood. He estimated that half of the $O_2$ partial pressure ($P_{O2}$) driving force between RBC interior and distal tissue is dissipated in the blood. From spectroscopic measurements of $O_2$ binding to myoglobin in cryogenically frozen tissue, Honig et al. (1984) deduced that muscle $P_{O2}$ ranges from 1 to 3 mmHg. This implies a much greater intracapillary $P_{O2}$ gradient and thus a more substantial fraction of resistance to $O_2$ transport being located in the blood phase than previously believed.

Although the true capillaries, blood vessels with diameters of 10 µm and less, are considered to be the primary exchange location; it has been observed that 25-30% of the microcirculatory gas transport occurs in the arterioles and larger vessels, 20-100 µm in diameter (Popel and Gross, 1979; Ivanov et al., 1982; Roth and Wade, 1986; Pittman, 1987). In addition, membrane oxygenators used in cardiopulmonary bypass surgery and in membrane lungs involve oxygen transport from relatively large conduits (about 100 µm and larger); and in most of these devices, similar to the in vivo situation, the mass transfer resistance attributable to the blood phase is a significant fraction of the total resistance. To design and evaluate increasingly efficient artificial lungs, it is necessary to understand the reactive, diffusive and convective mechanisms of $O_2$ and CO$_2$ transport in flowing blood. Therefore, it is important that we develop general models for describing intracapillary oxygen transport which are valid over a wide range of vessel diameters; for vessels with diameters of 20 up to several hundred microns, as well as for capillaries. This work focuses on the transport phenomena in the large vessels (20 -
several hundred microns in diameters) where the mass transfer and hydrodynamic characteristics are known to be different from those in the small capillaries.

The mechanisms of gas transport to and from different sizes of vessels in the microcirculation are different; the steps involved are the same, but the distributions of resistance are dissimilar. In large vessels (diameters of 20 μm and greater), most of the resistance lies in the plasma phase; in the intermediate diameter range, the intracapillary resistance within the red cells becomes comparable to that in the plasma; and in the capillaries (diameters of 8 μm and less), most of the resistance lies in the red cell phase (Nair et al., 1989). In true capillaries with diameters of 5 μm or less, RBCs are restricted to a single-file flow, and they tend to fold along a major axis which is parallel to the vessel axis. In larger capillaries, RBCs are observed to be travelling in either a single- or multi-file pattern depending on tube hematocrit and vessel diameter, and they often take on nonaxisymmetric shape which is resulted from a shift of internal hemoglobin solution from the trailing-edge into the leading-edge part of the cell (Cokelet, 1987). In still larger vessels, the RBCs more or less retain their disc shape, and there is a radial distribution of RBCs with higher hematocrit at the center and lower near the walls, and cell deviations from straight stream lines are somewhat suppressed (Gaehgens et al., 1980) However, in even larger vessels, the vessel walls do not impose such constraint on the cell motion, and when the blood is subject to shear flow, its formed elements undergo almost random motion. Various investigators (Colton and Drake, 1971; Dorson and Voorhees, 1974; Diller et al., 1980; and Wang and Keller, 1985) had reported that these shear-induced particle migrations and the associated fluid motion can significantly augment transport of oxygen under certain circumstances. Various processes involving in the gas transport and pH regulation by blood and the hydrodynamic characteristics of RBCs flowing in the microvessels are discussed in Chapter 2.
Considerable attention has been focused on the theoretical description of O₂ transfer to and from blood, and several reviews have appeared on the subject: Spaeth (1973), Artigue (1980), Nair (1988), and Popel (1989). The general convective-diffusive mass balance has been employed to describe gas transfer to and from blood. A rigorous application of such theory would be exceedingly complex, and several assumptions and approximations are necessary. Some investigators (Reneau et al., 1967; Buckles et al., 1968; Weissman and Mockros, 1969; Bradley and Pike, 1971; Villarroel et al., 1971; Benn et al., 1975; Voorhees, 1976; Diller et al., 1980) have simplified the problem by treating the blood as a continuous and homogeneous hemoglobin solution; while others (Hellums, 1977; Baxley and Hellums, 1983; Artigue and Bruley, 1983; Nair et al., 1989) have attempted to take into account the discrete nature of the blood. For tubes of large diameter, over 300 µm, where the characteristic radial length is much larger than the red cell size, the continuum approach has proven satisfactory (Weissman and Mockros, 1969; Bradley and Pike, 1971; Villarroel et al., 1971; Voorhees, 1976; and Diller et al., 1980). The flow in smaller vessels (arterioles and venules) is characterized by a nonuniform hematocrit distribution, at least by a cell-depleted plasma layer near the tube wall which is about 2 - 4 µm and is, in this situation, a considerable fraction of the entire tube diameter; and the characteristic radial length approaches that of the RBC size. Therefore, the applicability of the continuum models becomes questionable in these small microvessels. A brief review on the development and validation of the continuum models is included in Chapter 3.

Various investigators have used different sets of assumptions to reduce the complex governing equations to a tractable set. It has been difficult to critically test the assumptions of the various models, because experimental measurements of sufficient detail and accuracy are not readily available. Several prior workers have reported experimental data obtained from membrane oxygenator whose diameters range from 300
μm and up (Buckles et al., 1968; Weissman and Mockros, 1969; Bradley and Pike, 1971; Villarroel et al., 1971; Voorhees, 1976; Diller et al., 1980). However, until recently there has been insufficient experimental validation of the various assumptions used for the microvessels (vessels with diameters less than 100 μm). Boland et al. (1987) developed an in vitro microvessel microspectrophotometer system which allowed accurate determination of oxygen fluxes to and from RBC suspensions in small cylindrical conduits (diameter = 30 μm) under physiologically relevant conditions. In this system flow, transport and geometrical parameters can be controlled and measured accurately. Thus, validation of the various proposed models for application to the small microvessel has become possible.

Nair et al.'s (1989) discrete model for calculating O₂ transport rates in blood flowing through microvessels is entirely predictive. That is to say all parameters in the model were determined from the literature or by other means independent of oxygen transfer experiments. The model was shown to be in excellent agreement with the experimental oxygen transport results from 27-μm-diameter artificial membrane tubes (Boland et al.'s system), as well as with deoxygenation results obtained by Schmulker and Chien (1985) in 100-μm-diameter membrane tubes. Prior mathematical models were shown to be significantly less successful. Detailed calculations of the O₂ concentration distribution from Nair et al.'s model revealed that the dominant resistance to O₂ transport is distributed in the plasma and relatively little resistance is present within or in the immediate vicinity of the RBCs. Using the above results as a guide, Nair et al.'s model can be simplified from four simultaneous nonlinear partial differential equations (PDEs) to one PDE by introduction of a set of well-founded assumptions. The development of this simplified, discrete model and comparison of the two models are presented in Chapter 4. Other objectives of Chapter 4 include mapping out the
regime of applicability for both the continuum and discrete models and consideration of shear-induced augmented transport in blood.

While \( \text{O}_2 \) and \( \text{CO}_2 \) transfer are of comparable importance and the coupling between the two processes is significant, most prior analyses have focused on \( \text{O}_2 \) transfer. In general, a lesser extent of effort and attention has been paid to the blood's companion task of stripping \( \text{CO}_2 \) from tissue, eliminating it in the lung and to its influence on the overall mass transfer rate. Indeed, in many investigators' papers, artificial lungs are usually called oxygenators, as if their only task was blood oxygenation. Previous attempts on modeling the coupled transport have been along a semi-empirical and lumped-parameter approach. The majority of these theories treated blood as a homogeneous fluid. This approach has proved to be valid for the description of \( \text{O}_2 \) transfer in macro channel devices but not necessary appropriate for small microvessels. Although it was suggested by several investigators (Weissman and Mockros, 1969; Bradley and Pike, 1971; Dorson \textit{et al.}, 1971; Villarroel \textit{et al.}, 1971; Benn \textit{et al.}, 1975; Voorhees, 1976) that this continuum assumption may be valid for \( \text{CO}_2 \) transfer modeling under the conditions of low flow rates and large channel dimensions, typical of commercial oxygenators; the validity of this approximation over a range of diameters is questionable. Additionally, local chemical equilibrium was generally assumed. This assumption overlooks some of the more subtle aspects of \( \text{CO}_2 \)-blood interactions. These interactions include the synergistic actions between hemoglobin bound \( \text{O}_2 \) and \( \text{CO}_2 \) and the fact that the reaction scheme for \( \text{CO}_2 \) is much more complex than that depicted by the \( \text{CO}_2 \) dissociation curve. More importantly, the \( \text{CO}_2 \) hydration/dehydration reactions in the plasma are definitely far from being at equilibrium. Another problem in the use of this simplified equilibrium assumption arises in interpretation of the diffusive flux. Total \( \text{CO}_2 \), as defined by the equilibrium treatment, is a combination of bicarbonate ion (\( \text{HCO}_3^- \)), carbamino-\( \text{CO}_2 \) and dissolved
CO₂. As a result, adjustments for the effects of the individual species and the heterogeneity of blood have to be incorporated into the definition of an effective diffusivity. The interpretation of this effective property is not completely straightforward because of the highly complex nature of blood. Finally, it should be mentioned that most prior analyses have not treated the interrelationship of O₂ carriage in the blood to the amount of CO₂ in the blood (the Bohr effect) and CO₂ storage to the amount of O₂ in the blood (the Haldane effect). A literature review on the models for coupled O₂ and CO₂ transport is given in Chapter 3.

In order to investigate the coupling between the O₂ and CO₂ transport, an extended model is developed to incorporate the study of CO₂, as well as, a study of the various blood buffer systems that are closely connected to the transport of these gases. The blood is treated as two continuous coexisting phases: a RBC phase and a plasma phase. The microvessel is divided into two regions: the central, RBC-rich and the outer, cell-free region. The radial distribution of RBCs, and transport of various species due to bulk convection are taken into account. Chemical and transport processes which are included in the model are (1) association and dissociation of O₂ with hemoglobin, (2) association and dissociation of CO₂ with hemoglobin, (3) the Bohr and Haldane effects (the interdependence of O₂ and CO₂ transport), (4) CO₂ hydration/dehydration reactions, (5) buffering actions of hemoglobin and plasma proteins, and (6) anion exchange across the RBC membrane via an anion transporter. The anion transporter is a transmembrane protein that catalyzes the one-for-one exchange of two monovalent anions in opposite directions across the red cell membrane. The monovalent anions are bicarbonate, produced by the hydration of CO₂, and chloride. The exchange of these anions by the anion transporter has been shown to be essential to the respiration of CO₂. It is, therefore, important that we are able to describe this exchange system. The formulation and validation of a simplified kinetic model for the anion transporter is presented in
Chapter 5. Finally, the governing equations of the complete transport model subjected to the imposed inlet and boundary conditions are derived and solved numerically in Chapter 6 to provide the concentration distributions of various species in blood that are important in the O$_2$ and CO$_2$ exchange process.

The objectives of this work include the followings:

1. To review and discuss phenomena, including the blood gas chemistry and rheology, that are important for blood gas transport and pH regulation by flowing blood.

2. To conduct a literature survey on the existing models for describing the coupled O$_2$ and CO$_2$ transport phenomena.

3. To formulate a simpler and workable mathematical model for predicting O$_2$ transport rates by blood flowing in microvessels based on the model of Nair et al. (1989).

4. To investigate the feasible diameter regime for application of several existing models (both continuum and discrete models) for prediction of O$_2$ transport in vessels with diameters ranging from 20 up to several hundred μm.

5. To assess the importance of shear-induced augmentation on O$_2$ transport as a function of vessel diameter, shear rate and O$_2$ storage potential of the blood.

6. To develop a simplified model for describing the anion exchange across the RBC membrane based on the available literature information on this protein; to test the validity of the flux expression with available experimental data; and to incorporate this anion exchange model into an extended model of O$_2$ and CO$_2$ transport.

7. To incorporate CO$_2$ transport and pH regulation by blood into the O$_2$ transport model so that we will be able to further elucidate the inter-dependence of blood gas transport and acid/base balance and to validate the model with data from the literature.
CHAPTER 2

PHYSICAL SITUATION OF GAS TRANSPORT
AND BLOOD RHEOLOGY

2.1 General Description of Blood-Gas Interaction

Blood is a heterogeneous fluid composed of both a continuous plasma phase with colloidal suspended proteins and suspended cells. Plasma is a complex aqueous fluid that contains various inorganic ions, proteins, and organic substances; it is composed of 90% water and about 7% protein. Among the major protein constituents of plasma are albumins and globulins which maintain osmotic balance and thereby control the movement of water between blood and various tissues. Some globulins serve as protection against disease. Fibrinogen is a protein which plays an important role in the clotting mechanism of blood. Some blood proteins act as buffers for the blood.

There are about 5x10^9 cells in a milliliter of human blood. About 0.2% of the cells are white blood cells, or leukocytes, which protect the body against invasion by foreign micro-organism (Middleman, 1972). About 5% of the cells are platelets, or thrombocytes, which perform a function related to blood clotting. The red blood cells (RBCs), or erythrocytes, by far the largest volume of the formed elements, play the important dual roles of

1. Reversibly binding O_2 in the lungs and distributing it throughout the body for cellular metabolism;
2. Removal of CO_2 formed by metabolic processes from the tissues and transporting it to the lung where it is eliminated. The RBC is composed of about 72% water and 25%
hemoglobin (by volume). The rest of the cell consists of protein, mostly associated with
the structure of the cell membrane, and ions such as bicarbonate ($\text{HCO}_3^-$), chloride ($\text{Cl}^-$),
sodium ($\text{Na}^+$) and potassium ($\text{K}^+$).

While $\text{O}_2$ is carried by the blood mainly through a reversible chemical reaction
with hemoglobin, the transport of $\text{CO}_2$ involves a complex interaction of many
phenomena. The chemical and transport events that occur in blood during gas exchange
in a lung capillary (excluding the transcellular water movement) are shown schematically
in Figure 2.1 (modified from Klocke (1987) and Nunn (1987)). Reactions 1, 2 and 3
represent $\text{O}_2$ diffusion across the alveolar capillary membrane, diffusion into the RBC
and chemical reaction with intracellular hemoglobin molecule. Under normal conditions
in human circulation, greater than 95% of $\text{O}_2$ is reversibly bound to hemoglobin; and the
remaining $\text{O}_2$ is in a free form, dissolved in both blood plasma and in the hemoglobin
solution inside the RBCs. The remaining reactions in the respiration diagram deal with
$\text{CO}_2$ transport and $\text{pH}$ regulation. Under normal physiological conditions, roughly 0.7
$\text{H}^+$ (Bohr protons) are released from intracellular hemoglobin every time an $\text{O}_2$ molecule
is bound (reaction 4). In native RBCs, these acid equivalents rapidly equilibrate with
$\text{HCO}_3^-$ in the presence of carbonic anhydrase (represented as CA in Figure 2.1) to
produce $\text{H}_2\text{O}$ and $\text{CO}_2$. Free $\text{CO}_2$ then diffuses out of the cell rapidly since plasma
membrane is highly permeable to apolar gases (reaction 6). $\text{CO}_2$ persists in the plasma
phase and then is exchanged into the alveolus (reaction 7). Therefore, reactions 5-7
allow intracellular $\text{HCO}_3^-$ to be expelled as $\text{CO}_2$ in the lung. However, the bulk of
venous $\text{HCO}_3^-$, about 80%, is present initially in the plasma. In the absence of
extracellular carbonic anhydrase, the $\text{CO}_2$ dehydration reaction (reaction 8) occurs much
too slowly to be of importance. Therefore, in order for these extracellular $\text{HCO}_3^-$ to be
evolved as $\text{CO}_2$ from lung capillaries, it must be transported across the RBC membrane
(reaction 9). This involves one-for-one exchange of $\text{Cl}^-$ for $\text{HCO}_3^-$ to maintain
Figure 2.1: Exchange of oxygen and carbon dioxide in a pulmonary capillary.
electroneutrality, and it is mediated by the anion transporter (AT). As result, 81% of CO₂ is carried along with the plasma and RBCs from the tissue to the lung as HCO₃⁻. The other mechanism which CO₂ is carried by the blood is through direct reversible chemical combination with the N-terminal valines of α and β chains of hemoglobin (reaction 10). In this form, 11% of the CO₂ is transported by the blood to the lung. Finally, dissolved CO₂ accounts for 8% of CO₂ being transported to the lung. In the case of the O₂ and CO₂ exchange between the blood and the respiring muscle tissues, the same processes occur in the reverse direction.

2.1.1 Carriage of Oxygen in Blood

O₂ dissolves physically throughout in the blood, but over 95% of the available O₂ is transported principally through reversible combination with the protein hemoglobin. A hemoglobin molecule is made up of four subunits united in a tetrameric conformation as illustrated in Figure 2.2 (taken from Lehninger, 1975). Each subunit contains a heme moiety conjugated to a polypeptide. The polypeptides are referred to collectively as the globin portion of the hemoglobin molecule. There are two pairs of polypeptides in each molecule, two of subunits containing one type (alpha chains) and two containing another (beta chains). The alpha (α) subunits and beta (β) subunits pair to form asymmetric dimers, denoted arbitrarily, α₁β₁ and α₂β₂. Therefore, these dimers of unlike chains are the fundamental structural units of the hemoglobin tetramer, and in the RBC, the hemoglobin molecules continually dissociate into dimers and reassociate into tetramers (Baumann et al., 1987).

The hemoglobin molecule and its subunits contain mostly hydrophobic amino acids internally and hydrophilic amino acids on their surface. Therefore, the molecule is soluble in water but impermeable to water. The heme prosthetic groups are in the four largely hydrophobic pockets, one being formed in each globin peptide chain
Figure 2.2: The quaternary structure of hemoglobin (copied directly from Lehninger (1975)).

(Figure 2.2). Heme is an iron-containing porphyrin derivative; it is the active center of the hemoglobin molecule, the binding site for O₂. The non-aqueous environment around the iron atom allows it to remain in the ferrous state even in the presence of O₂ molecules, and imparts to the iron-oxygen bond a coordination character that makes it strength intermediate between a non-covalent and a covalent bond. This property is important in allowing the reversibility of O₂ binding. The four interacting subunits of hemoglobin molecule generate a cooperative effect. The positive cooperative effect of the tetrameric hemoglobin can be described as follows: if a hemoglobin molecule takes up one O₂, it tends to go on and acquire two or three more O₂ molecules; and vice-versa, hemoglobin’s affinity for O₂ decreases with decreasing O₂ saturation. Hemoglobin is a protein molecule that changes its structure in response to chemical stimuli such as the O₂ molecule; it is known that the stable quaternary structure of the oxygenated hemoglobin is significantly different from that of the deoxygenated form (Perutz, 1978). Additionally, the striking feature of protein chemistry in relation to O₂-
hemoglobin interaction is allostery - the coupling of ligand (an organic molecule that donates the necessary electrons to form coordinate covalent bonds with metallic ions, as O\textsubscript{2} is bound to the central iron atom of hemoglobin) binding at one protein site to change in the conformation of the macromolecule due to the binding at a second site.

2.1.1.a Oxygen Hemoglobin Equilibrium

The O\textsubscript{2}-binding characteristics of hemoglobin can be described by a curve, oxyhemoglobin dissociation curve (ODC), obtained by plotting the fractions of available hemoglobin sites saturated with O\textsubscript{2} (S) as a function of the partial pressure of O\textsubscript{2} (P\textsubscript{O2}) in equilibrium with the solution. One way of characterizing the O\textsubscript{2} affinity of hemoglobin consists of determining P\textsubscript{50}, which is the P\textsubscript{O2} leads to 50% saturation. For example, at 37 °C and pH 7.4 (physiological conditions), P\textsubscript{50} for intraerythrocytic hemoglobin is about 26 mmHg. As the hemoglobin O\textsubscript{2} affinity is decreased (P\textsubscript{50} is increased), relatively less O\textsubscript{2} is bound to hemoglobin; or alternatively, a greater P\textsubscript{O2} is needed to bind the same amount of O\textsubscript{2}. Another way to describe the behavior of hemoglobin towards O\textsubscript{2} is to characterize the degree of sigmoidicity of the ODC. The sigmoidal shape of this curve reflects the cooperative effect of O\textsubscript{2} binding to hemoglobin, which is the result of conformational changes of different subunits during the oxygenation or deoxygenation process. The sigmoid character of the ODC can be described quantitatively by Hill’s coefficient, n. When the cooperativity of hemoglobin is modified, this results in a decrease of Hill’s coefficient (e.g., n=1 for myoglobin, which binds O\textsubscript{2} in a non-cooperative manner, and n=2.5-3 for normal intraerythrocytic hemoglobin); and its value can thus be considered as a useful reflection of the efficiency of O\textsubscript{2}-carrying function (Lehninger, 1975). The sigmoidal nature of O\textsubscript{2} equilibrium curve itself contributes greatly to the efficiency of hemoglobin by causing a release of much O\textsubscript{2} over a narrow range of tissue P\textsubscript{O2} values (20-40 mmHg) and regaining maximal O\textsubscript{2} with a limited increase in P\textsubscript{O2} (100 mmHg) as it returns to the lung.
Many physicochemical conditions within the blood determine, in concert, the actual values of $P_{50}$ and $n$. Several effectors or regulators of hemoglobin affect $O_2$ affinity and hence shift the ODC. For example, the curve is shifted right (increased $P_{50}$ or decreased $O_2$ affinity) with elevated $H^+$, $CO_2$, 2,3-diphosphoglycerate (DPG) and temperature (Hlastala, 1984). The dependence of the ODC on $CO_2$ concentration is known as the Bohr effect (Hlastala and Woodson, 1983). $CO_2$ is bound preferentially to deoxygenated hemoglobin, and therefore the $O_2$ affinity of hemoglobin decreases. However, only part of Bohr effect is due to molecular $CO_2$, the remainder being due to the presence of $H^+$, formed by the hydration of $CO_2$ and subsequent dissociation of carbonic acid. The latter effect is called the fixed acid Bohr effect which is determined when the $pH$ is changed by addition of fixed acid or base alone. Therefore, $CO_2$ lowers hemoglobin's $O_2$ affinity, not only by carbamino formation at the amino-terminal residues of alpha and beta chains, but also by its action in the form of $HCO_3^-$, an anionic effector of hemoglobin function. Briefly, Bohr effect enhances $O_2$ transport in blood in the following manner. As blood pass through the lung, $CO_2$ diffuses from the blood into alveoli. This reduces the partial pressure of $CO_2$ ($P_{CO_2}$) and the concentration of $H^+$ in blood. Both effects shift the ODC to the left and upward; so that the quantity of $O_2$ that binds with hemoglobin at any given $P_{O2}$ becomes considerably increased, thus allowing greater $O_2$ transport to the tissues. Then when blood reaches the respiring tissue capillaries, exactly the opposite effects occur. The $pH$ of blood passing through the capillaries drops continuously due to uptake of $CO_2$ and other acids; this displace $O_2$ from the hemoglobin and delivers $O_2$ to the tissue at a higher $P_{O2}$ than would otherwise occur. Thus Bohr effect is particularly important under the conditions of heavy muscular exercise.

Because the preferential binding of DPG, which is an erythrocyte metabolite intermediate, with the deoxygenated form of hemoglobin, the presence of DPG has
several forms of action. It has a direct effect on hemoglobin; binding of DPG to hemoglobin reduces the affinity to O₂ because it stabilizes the deoxygenated form of hemoglobin by cross-linking the beta chains and contributing additional salt bridges that must be broken for the deoxygenated form to click into the oxygenated form of hemoglobin (Martin, 1981). In addition, because the binding of DPG to the N-terminal (amino-terminal) valine of beta chains, the presence of DPG decreases the effect of CO₂. Several other organic and inorganic phosphates had also been found to have similar effects in vitro (Chanutin and Curnish, 1967). However, in human RBCs, only DPG and adenosine triphosphate (ATP) are present in sufficient quantity to influence the O₂ affinity of blood. However, the concomitant presence in human RBCs of a divalent cation such as Mg⁡²⁺, which binds to ATP to form an unreactive Mg-ATP complex, minimizes the allosteric effect of ATP on human hemoglobin. Moreover, 70 to 80% of intraerythrocytic ATP is bound to Mg⁡²⁺; therefore, it appears plausible to exclude ATP concentrations from the computations (Bunn et al., 1971). Finally, increased temperature has a direct effect on hemoglobin, reducing its affinity for O₂. It also decreases the fixed-acid Bohr effect and increases the effect of molecular CO₂. Increased temperature also decreases the binding of DPG to hemoglobin (Hlastala, 1984).

Such intricacy has precluded even a phenomenological model of blood O₂-hemoglobin equilibria at varying CO₂, acidity, DPG and temperature levels. However, several research terms have developed convenient representations of experimental data obtained over wide ranges of conditions, and these equations of state are discussed in more details in the following two sections. On the other hand, O₂ binding to hemoglobin also affects transport of other substances. Particularly, the dependence of CO₂ transport on O₂ concentration is called the Haldane effect which is the O₂-linked lowering of blood’s CO₂ affinity and is discussed in more details in a later section.
Together the Bohr and Haldane effects linked the problem of CO₂ transport intimately to the problem of O₂ transport, and the interactions result in facilitation of gas exchange at the lung and in the systemic tissue.

2.1.1.b *Equations for Standard Oxyhemoglobin Dissociation Curve*

An excellent review on modeling blood ODC was published by O’Riordand and Colleagues (1985) in which they compare nine different models fitted to normal human data. Some of these models are summarized in Table 2.1. It is not the intent here to review all models for the ODC, but rather to compare them. Among many models for ODC, the empirical Hill (1910) and the more theoretical Adair (1925) equations are best known. The Hill model gives a good characterization over the saturation range 20-98% which is the range of major physiological interest. The significance of the Hill parameters, $P_{50}$ and $n$, is well known; $P_{50}$ reflects the affinity of hemoglobin molecule toward O₂, and $n$ is an empirical index of cooperativity of O₂ binding to hemoglobin. Under standard conditions ($T=37$ °C, $pH=7.4$, $P_{CO₂}=40$ mmHg and the molar ratio of $[DPG]/[Hb₄]=0.9$ where Hb₄ denotes hemoglobin), it has been reported that the mean $P_{50}$ is 26.7±1.7 mmHg and $n$ is about 2.6-2.8 (O’Riordan *et al.*, 1983; Winslow *et al.*, 1983).

Adair’s stepwise hypothesis paved the way for modern conception of the hemoglobin molecule. He proposed that four O₂ molecules bind to a single hemoglobin molecule sequentially, giving four equilibrium constants, one for each sequential reaction. This type of equation is difficult to fit because the parameters are rather closely correlated (i.e., the parameters are redundant) (Winslow *et al.*, 1977; Fell, 1979). Margaria (1963) simplified the Adair model by equating the first three equilibrium constants because they were of similar magnitude while the forth was much higher. $k$ and $η$ of the of the Margaria approximation are constants; the value of $η$ is generally given as 125 while $k$ is a function of $P_{50}$ ($k=0.0124$ when $P_{50}=27$ mmHg). The
Margaria model with its two parameters would certainly be easier to fit than the four-parameter Adair model from whence it was derived.

**Table 2.1**: Models describing the oxygen-hemoglobin equilibrium curve.

<table>
<thead>
<tr>
<th>ODC Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill (1910)</td>
<td>[ S = \frac{(P_{O2}/P_{50})^n}{1 + (P_{O2}/P_{50})^n} ]</td>
</tr>
<tr>
<td>Adair (1925)</td>
<td>[ S = \frac{a_1P_{O2} + 2a_2P_{O2}^2 + 3a_3P_{O2}^3 + 4a_4P_{O2}^4}{4(1 + a_1P_{O2} + a_2P_{O2}^2 + a_3P_{O2}^3 + a_4P_{O2}^4)} ]</td>
</tr>
<tr>
<td>Margaria (1963)</td>
<td>[ S = \frac{(\kappa P_{O2})^3 + \eta - 1}{(\kappa P_{O2})^4 + \eta - 1} ] where ( \kappa P_{O2} = \frac{1 + k P_{O2}}{k P_{O2}} )</td>
</tr>
<tr>
<td>Kelman (1966)</td>
<td>[ S = \frac{a_1P_{O2} + a_2P_{O2}^2 + a_3P_{O2}^3 + P_{O2}^4}{a_4 + a_5P_{O2} + a_6P_{O2}^2 + a_7P_{O2}^3 + P_{O2}^4} ]</td>
</tr>
<tr>
<td>Easton (1979)</td>
<td>[ S = (S_{max} - S_{min}) \exp[- \exp[\kappa(P_{O2}^* - P_{O2})]] + S_{min} ] where ( P_{O2}^* = \frac{\ln(E_c/\kappa)}{\kappa} )</td>
</tr>
</tbody>
</table>

Kelman (1966) used one of the more complicated equation for the ODC, a ratio of two polynomials, requiring seven parameters \((a_1-a_7)\) for standard conditions. Kelman completely generalized Adair equation parameters to allow greater flexibility; thus the model may be able to provide a better fit even through that it has no theoretical basis. Unfortunately, this also caused the parameter redundancy to reach unacceptable levels. Easton’s (1979) model for the ODC is based on a theoretical relationship between the partial pressure and equilibrium kinetics. The model requires two parameters: a rate coefficient \(E_c\) and a rate parameter \(\kappa\), and two scaling factors, \(S_{max}\)
and $S_{\text{min}}$, $S_{\text{max}}$ represents the maximum (upper) saturation scale, and $S_{\text{min}}$ represents the minimum (lower) saturation scale. The Easton lumped parameter $E_{lp}$, which is a ratio of $(E_c/\kappa)$ can be shown to be a grouping of the two scaling parameters such that:

$$E_{lp} = \ln[(S_{\text{min}} - S_{\text{max}})/S_{\text{min}}].$$

The $E_{lp}$ factor describes the leftward or rightward shift of the ODC, much like the Hill parameter $P_{50}$; additionally, his parameter $\kappa$ is supposed to determine the slope of the ODC, like the Hill parameter $n$.

As a result of O'Riordan et al.'s (1985) investigations, they reported that for most applications, the Hill equation still appears to give an adequate characterization over the physiologically important part of the ODC. As expected, when compared with the Hill model, an increase in accuracy was obtained with the models such as Adair, Kelman and Easton. Although these models are also applicable over the complete range of saturation, this extra information is in their opinion of little physiological interest. In addition, they also suggested that when the most accurate description of the ODC is required, as when looking for small changes in ODC, the Easton model would appear to be the best choice. The three parameters are relatively easy to determine although the slightly higher parameter redundancy necessitates good initial parameter estimates. Independently, Buerk (1985) evaluated the Easton model and found it to be superior to the Hill equation and comparable to the Adair equation in the saturation range from 0 to 95%. Reich and Zinke (1984) also examined the parameter redundancy for different models. They found that the Kelman model fit their data best. However, the uncertainty in the seven parameters was large. They found some parameter redundancy with all models and suggested that the simpler ODC may ultimately be the most useful.

2.1.1.c  Equations for Nonstandard Oxyhemoglobin Dissociation Curve

Understanding the gas exchange processes can not be accomplished without detailed empirical knowledge of hemoglobin, oxygen and effectors in interactions in the blood. Therefore, it is necessary that we are able to describe $O_2$-hemoglobin
equilibrium relationship and the important aspects of how ODC varies with $P_{CO2}$, $pH$, DPG and temperature. It is essential that we are able to compute the full range of the curve; such that if $P_{O2}$, $pH$ and $P_{CO2}$ are given, $S$ can be calculated; this is to account for the Bohr effect which is attributed to $pH$ changes, due to CO$_2$, and consequent allosteric effects. It is also important that we understand the allosteric effect of DPG on hemoglobin. It is well known that in some pathological and adaptive conditions DPG concentration within the RBC can vary widely. Some of these conditions are anaemia, hexokinase and pyruvate kinase deficiencies, acidosis, shock, massive blood transfusion, congenital and acquired heart disease, chronic lung disease, and high altitude adaptation (Sarnaja and Winslow, 1979).

Arturson et al. (1974) tabulated the “effector ligand coefficients”,

$$\left( \frac{\partial \log P_{O2}}{\partial \log X_i} \right)_{S, x_{j \neq i}} \text{ where } X_i = [H^{+}], P_{CO2} \text{ or } [DPG].$$

The use of such coefficient is motivated by the analysis of “allosteric linkage” by Wyman (1964). Wyman derived “reciprocal relationships” - comparable to Maxwell’s relations in thermodynamics - such as the following:

$$\left( \frac{\partial [H^+]}{\partial [HbO_2]} \right)_{pH} = -\left( \frac{\partial \log P_{O2}}{\partial pH} \right)_{S}$$  \hspace{1cm} (2.1)

The derivative on the right is called the “Bohr coefficient” which is a measure of change in O$_2$ affinity with $pH$. On the left of Equation (2.1) is the “Haldane coefficient” which is a measure of release or gain of protons accompanying oxygenation or deoxygenation. Tyuma and Ueda (1975) also experimentally substantiated this theoretically derived equivalence of the Bohr and Haldane coefficients in a wide range of O$_2$ saturation.
Samaja et al. (1981) developed an empirical method for the ODC variation with pH, $P_{CO_2}$ and $[DPG]/[Hb_4]$ molar ratio based on Hill’s equation. They examined how the Hill parameters varied by fitting human blood data obtained under a variety of conditions, and the effects of temperature were not determined. Because $\log P_{50}$ was observed to be a linear function of $pH$ in the range of 6.9 to 7.6, its value at any $pH$ in this range can be interpolated by use of the following formulas (Samaja et al., 1981):

$$\log \text{log } P_{50}(pH) = \frac{(pH - 7.0)[\log P_{50}(pH=7.6) - \log P_{50}(pH=7.0)]}{0.6} + \log P_{50}(pH=7.0)$$ \hspace{1cm} (2.2a)

$$\log P_{50}(pH=7.0) = (B_1 P_{CO_2} + B_2) \left[\frac{[DPG]}{[Hb_4]}\right] + B_3 P_{CO_2} + B_4$$ \hspace{1cm} (2.2b)

$$\log P_{50}(pH=7.6) = (B_5 P_{CO_2} + B_6) \left[\frac{[DPG]}{[Hb_4]}\right] + B_7 P_{CO_2} + B_8$$ \hspace{1cm} (2.2c)

where $B_1=-6.912x10^{-4}$, $B_2=0.3365$, $B_3=3.598x10^{-4}$, $B_4=1.599$, $B_5=-1.380x10^{-3}$, $B_6=0.3607$, $B_7=9.089x10^{-4}$, $B_8=1.360$; $P_{CO_2}$ is in mmHg, and $[DPG]/[Hb_4]$ is a molar ratio. It should be mentioned that Samaja et al.’s empirical relationship allows the estimate of normal human whole blood $P_{50}$ at any given $pH$ (range 6.9 to 7.6), $P_{CO_2}$ (range 20 to 90 mmHg), and $[DPG]/[Hb_4]$ ratio (range 0.3 to 2.5), with 0.73 mmHg SD. Together with the above, it was also assumed that Hill’s factor, $n$, would be approximately constant under various conditions of $pH$ and $[DPG]/[Hb_4]$. Therefore, the Bohr effect and organic phosphate (DPG) effect on the ODC can be accounted for through the fact that $P_{50}$ is now a function of $P_{CO_2}$, $pH$ and DPG.

However, characterization of the ODC by Hill’s approximation does not account for changes in shape under various conditions of $pH$ and $[DPG]/[Hb_4]$; consequently, Winslow et al. (1983) set out to correlate not only the position but also the shape of the curve to the values of the various effectors. They developed an algorithm for the
oxygen saturation with $pH$, $P_{CO2}$ and DPG based on Adair's equation because the four Adair parameters appeared to be sensitive to these effects. In order to generate the nonstandard ODC, they examined how the Adair model parameters ($a_i$; $i=1, 2, 3, 4$) varied by fitting human blood data under a variety of conditions. The resulting set of Adair parameters were then empirically fit to quadratic equations, requiring a total of 72 coefficients. The temperature effects were not determined, but would require that an additional 24 coefficients be estimated in order to extend this algorithm. Their data base were more extensive than had previously been available: $P_{O2}$ up to 150 mmHg, $pH$ between 7.2 and 7.8, $P_{CO2}$ between 7 and 70 mmHg, and [DPG] between 1 and 14 mM. Kelman (1966) determined the effect of temperature variation and used the following empirical relationship, requiring three coefficients, given by

$$P_{O2,S} = P_{O2,NS} \left[0.024 (37 - T) + 0.4 (pH - 7.4) + 0.06 \log \left(40/P_{CO2}\right)\right]$$

(2.3)

where subscripts "S" and "NS" denote standard and nonstandard conditions, respectively; and $T$ is in °C and $P_{CO2}$ is in mmHg. The effects of DPG were not included, since its effects were not known at the time. Saturation can then be computed after correcting the nonstandard $P_{O2}$ to standard conditions and substitute into a standard ODC.

The Easton model was shown to be comparable to the Adair equation; however, the Easton model involves only two parameters and a scaling factor while Adair model requires four parameters. In addition, Easton's model offers a computational advantage, since explicit mathematical equations can be written for either oxyhemoglobin saturation as a function of $O_2$ partial pressure, or vice versa; and numerical methods are required to invert Adair's equation. Furthermore, Easton (1979) had shown that only one of the model parameters will vary with nonstandard conditions, which would simplify
calculations for nonstandard ODC. Recognizing these advantages, Buerk and Bridges (1986) modified Easton’s model and developed an algorithm for which nonstandard ODCs can be computed. They rewrote Easton’s model by defining a new parameter, $P_{O_2}^*$, which is the $O_2$ partial pressure where the slope of ODC ($dS/dP_{O_2}$) is at maximum. They showed that the maximum slope for the Easton’s model occurs at

$$P_{O_2}^* = \frac{\ln \left( \frac{E_c}{K} \right)}{\kappa}$$  \hspace{1cm} (2.4)

Using their redefined parameter, the saturation is given by the double exponential (Buerk and Bridges, 1986):

$$S = (S_{max} - S_{min}) \exp \{- \exp \left[ \kappa \left( P_{O_2}^* - P_{O_2} \right) \right] + S_{min}$$  \hspace{1cm} (2.5)

As they had defined the model, the dimensionless product $\kappa P_{O_2}^*$ would not vary under nonstandard conditions. $P_{O_2}$ can be computed explicitly from

$$P_{O_2} = \frac{\left( \kappa P_{O_2}^* - \ln \left[ \ln \frac{S_{max} - S_{min}}{S - S_{min}} \right] \right)}{\kappa}$$  \hspace{1cm} (2.6)

They estimated the Easton parameters for the standard human ODC (@ $T=37$ °C, $pH=7.4$, $P_{CO_2}=40$ mmHg and $[DPG]/[Hb_4]=0.9$) from ten previously reported human blood data in the literature. Overall, the mean values (±SE) were $\kappa=0.0725±0.0059$ (mmHg)$^{-1}$ and $P_{O_2}^*=20.70±0.30$ mmHg. By restricting the fit to data in the saturation range from 0 to 95%, they found an upper scaling factor $S_{max}=96.01±0.22\%$ and lower scaling factor $S_{min}=1.23±0.23\%$. It should be mentioned that the corresponding $P_{S_0}$ is 26.8 mmHg for those ten data sets. They then determined how $P_{O_2}^*$ and $\kappa$ vary under
nonstandard conditions by comparing the modified model with two previous nonstandard ODC algorithms (Kelman, 1966 and Winslow et al., 1983) in the literature. They found that the conditions can be characterized by additional four parameters. The rate constant $\kappa$ for the modified Easton model varies with all four factors as

$$
\kappa_{NS} = \kappa_S \exp\left[B_1(pH \cdot 7.4) + B_2(PO_2 \cdot 40) + B_3(T \cdot 37) + B_4\sqrt{[DPG]/[Hb_4]} - 0.9\right]
$$

(2.7)

where subscripts "S" and "NS" denote standard and nonstandard conditions; $B_1$=0.765 (pH)$^{-1}$, $B_2$=-1.47x10$^{-3}$ (mmHg)$^{-1}$, $B_3$=-0.0611 (°C)$^{-1}$ and $B_4$=-0.291. The applicable range for the description of ODC is for $PO_2$ 0-150 mmHg, $T$ 20-40 °C, $pH$ 6.8-8.0, $PCO_2$ 7-70 mmHg, and $[DPG]/[Hb_4]$ 0.6-2.0.

Because this model also describes hemoglobin solutions as well as whole blood (Easton, 1979), it would also be useful for characterizing O$_2$ transport and delivery with hemoglobin-based artificial blood substitutes. New estimates for the model parameters ($\kappa$, $PO_2^*$, $S_{max}$) and nonstandard coefficients ($B_1$-$B_4$) would be required for the specific blood substitute. This modified Easton algorithm would be superior than Samaja's modified Hill model for the obvious reason that it is of adequate accuracy over a variety of conditions; while the modified Hill model accounts for changes in shifting but not in shape of the dissociation curve under various conditions. It also offers computational advantages over the previous algorithms by Kelman (1966) and Winslow et al. (1983). First, explicit forms of $S=S(PO_2)$ and $PO_2=PO_2(S)$ are readily obtainable (Equations (2.5) and(2.6)), avoiding the iterative numerical procedures that are required to invert either Adair or Kelman's equations. Second, less computer time is required to compute new parameters for nonstandard conditions, since only one parameter is involved.
2.1.2 Carriage of Carbon Dioxide in Blood

The uptake and release of CO₂ take place during the passage of the blood through tissue and lung capillaries, respectively. The processes occurring in the lung capillaries are depicted schematically in Figure 2.1. Under normal circumstances, only about 5% of the total CO₂ content of blood is carried as free CO₂. Predominantly, CO₂ is found in blood chemically combined into two species: as bicarbonate, the primary CO₂ carriers in both plasma and RBCs, and as carbamino - CO₂ in combination with amino groups on proteins, particularly as hemoglobin carbamate. The portions of different form of CO₂ depends largely upon the acid/base status of the blood. While the vast bulk (typically about 97%) of O₂ is carried by hemoglobin inside the RBCs, CO₂ is somewhat more evenly distributed between the blood phases. Plasma HCO₃⁻ is normally the major CO₂-bearing species because of the greater plasma volume, CO₂ solubility and pH. Because the volume fraction occupied by water is 0.94 in plasma and 0.72 in the cells; solubility coefficient of CO₂ is 0.031 mM/mmHg in the plasma, while it is 0.023 mM/mmHg in the RBC. With a normal hematocrit of 0.40-0.45, plasma transports about two-thirds of all CO₂ carried in blood.

The simple shape of the CO₂ dissociation curve (Figure 2.3) is deceptive, since the reaction scheme for CO₂ is far more complex than that for O₂, as illustrated in Figure 2.1. For the case which CO₂ is removed in the lung or with an artificial membrane oxygenator, bicarbonate and hydrogen ions form carbonic acid which is then decomposed to liberate CO₂. However, the dehydration reaction of carbonic acid to form CO₂ in the plasma is very slow, thus HCO₃⁻ is the predominant species. HCO₃⁻ can translocate into RBC, although slowly, in exchange of of other anions, in particular Cl⁻, where the same chain reactions then occur. In the RBC, however, dehydration of carbonic acid is catalyzed by the enzyme carbonic anhydrase. The predominant mode for liberation of CO₂ from blood is therefore translocation of bicarbonate into the RBC.
where it reacts with hydrogen ion to form carbonic acid. Dehydration of carbonic acid liberates CO₂ which, in turn, diffuses out of the RBC into the plasma, diffuses and convects within the bulk of the plasma, and then diffuses across the blood-membrane interface. Decomposition of carbamino hemoglobin is an additional source of CO₂. Carbamate compounds that arise from combination of CO₂ with plasma proteins have a much smaller effect because of the relatively unfavorable equilibria for their formation. Finally, various ionic species, such as organic and inorganic phosphates, amino acids, and proteins, behave as weak acids at physiological pH range. The buffering power of hemoglobin is particularly strong and has a marked effect in influencing the shape of the CO₂ dissociation curve.

![Graph](image)

**Figure 2.3:** Carbon dioxide dissociation curve of human blood at 37 °C plotted on a linear (A) and logarithmic (B) axes. [Copied directly from Klocke (1987)].
A carrier mechanism has been established in which O₂ and hemoglobin combine rapidly to form oxyhemoglobin which then diffuses in parallel with the diffusing O₂. The transfer of the O₂ is thus augmented or facilitated by the cooperative movement of oxyhemoglobin. For mathematical analysis of O₂ transport by flowing blood in microvessels, assumption of the reactants to be at chemical equilibrium, was shown to be a good approximation (Nair et al., 1990). This is due to the fact that in the microvessels (vessels with diameter of 30 μm and larger) the fraction of resistance to O₂ transport which is attributed to the RBC interior is very small, and to the fact that almost all O₂ is carried inside the RBC; thus the chemical equilibrium assumption is appropriate for the O₂ transfer problem. On the other hand, the transport of CO₂ through the bicarbonate solutions is enhanced by the hydration of CO₂. The hydrated CO₂ in the form of bicarbonate ion; diffuses along with dissolved CO₂. Intracellular hydration reaction is catalyzed by the carbonic anhydrase enzyme; thus the chemical equilibrium assumption would again be applicable. However, the extracellular hydration reaction is very slow, and the involving species are, in general, far from chemical equilibrium. Moreover, about two-thirds of CO₂ transport is carried out in the plasma region. Therefore, the simplifying approach of utilizing the CO₂ dissociation curve to describe the transfer of CO₂ might not be valid. This implies that perhaps one would not be able to consider the CO₂ transfer problem by considering the CO₂ dissociation curve alone; one would need to consider the intracellular and extracellular CO₂ transport events separately and then tie these processes together with interphase exchange of various species.

2.1.2.a *Bicarbonate System*

Carbonic acid and CO₂ are linked through the hydration/dehydration
\[ \begin{align*}
  &k_u \\
  &CO_2 + H_2O \leftrightarrow H_2CO_3 \\
  &k_v
\end{align*} \]

(2.I)

where \( k_u \) and \( k_v \) are the forward and backward reaction rate constants, respectively, for the hydration/dehydration reactions. Under normal circumstances, this reaction is extremely slow; however, in the RBC, the enzyme carbonic anhydrase greatly speeds up the rates of both forward and reverse reactions. Carbonic anhydrase is a zinc-containing enzyme of low molecular weight. It is inhibited by a large number of unsubstituted sulphonamides (general formula \( R-SO_2NH_2 \)). Acetazolamide is the most important of all these active inhibitors; this drug produces complete inhibition at 5-20 mg/kg in all organism and has no other pharmacological effects of importance. Acetazolamide has been much used in the study of carbonic anhydrase and has revealed the surprising fact that it is not essential to life. With total inhibition, \( PCO_2 \) gradients between tissues and alveolar gas are increased. Pulmonary ventilation is increased and alveolar \( PCO_2 \) is decreased (Kernohan, 1965; Dodgson and Forster, 1983; Bidani and Crandall, 1985). Otto and Quinn (1971) had shown that at enzyme concentrations approaching those found in the RBC the reaction rate is no longer linearly dependent on enzyme concentration but passes through a maximum followed by a region where the activity is independent of the carbonic anhydrase enzyme. Measurements of the catalytic activity of RBC hemolysates and suspensions have given a value of 6,500-15,000 for the catalytic factor inside the intact RBC (Itada and Forster, 1973; Dodgson and Forster, 1983).

At the \( pH \) of physiological significance, carbonic acid instantly dissociates into \( H^+ \) and \( HCO_3^- \).

\[ \begin{align*}
  &K_1 \\
  &H_2CO_3 \leftrightarrow H^+ + HCO_3^- \\
\end{align*} \]

(2.II)
where $K_1$ is the first acidic dissociation constant of carbonic acid. According to the law of mass action: $[H^+][HCO_3^-]/[H_2CO_3]=K_1$. The $pK_1$ is about 6.1; therefore, carbonic acid is about 96% dissociated under physiological conditions (Otto and Quinn, 1971). Bicarbonate can further dissociate into carbonate ion with liberation of a proton.

$$K_2$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}$$

(2.III)

where $K_2$ is the second dissociation constant of carbonic acid. However, the second dissociation occurs only at high $pH$ (above 9), because $pK_2 > 10$; and it is, therefore, not a factor in the carriage of $CO_2$ by the blood.

Interconversion of $HCO_3^-$ and $CO_2$ can also be achieved through the following pathway which involves the combination of hydroxyl ion with $CO_2$:

$$CO_2 + OH^- \Leftrightarrow HCO_3^-$$

(2.IV)

This reaction only becomes significant with increasing $pH$ because of the greater $OH^-$ concentration. Klocke (1987) calculated that at $pH$ 7.4 and 37 °C, only 9% of reaction product is formed via the hydroxyl pathway; and at $pH$ 7.2 and 7.6, this fraction becomes 6% and 14% respectively. Therefore, at $pH$ values less than 7.6; the contribution from Reaction (2.IV) is reasonably small and will be neglected in the analysis.

Consequently, the physiologically relevant pathways for hydration/dehydration reactions are Reactions (2.I) and (2.II). The rate of hydration of $CO_2$ per unit volume of aqueous media can be expressed as (Garg and Maren, 1972; Bidani et al., 1978)
\[- \frac{d[CO_2]}{dt} = \hat{A} \left( k_d [CO_2] - \frac{k_v}{K_1} [H^+] [HCO_3^-] \right) \]

(2.8)

where

$[CO_2]$ is the dissolved CO$_2$ concentration expressed in unit volume of aqueous media.

$[H^+]$ and $[HCO_3^-]$ are the concentrations of hydrogen and bicarbonate ions, respectively; and they are expressed in unit volume of aqueous media.

$\hat{A}$ is a parameter that varies with the presence of carbonic anhydrase activity. If the reaction is uncatalyzed, i.e., no carbonic anhydrase is present, then $\hat{A}=1.0$. The rate expression can be used for both the intra- and extracellular compartments. Extracellular carbonic anhydrase activity is thought to be normally absent ($\hat{A}=1.0$), but may be present if hemolysis did occur ($\hat{A}>1.0$).

There, however, had been some experimental evidences which indicated that a relatively small concentration of carbonic anhydrase is localized to the capillary endothelium in some tissues and can catalyze the plasma CO$_2$ hydration to some extent (Forster, 1982; Bidani and Crandall, 1985). In the present analysis, it will be assumed that carbonic anhydrase exists only in the RBCs and that plasma hydration is uncatalyzed. However, the analysis can readily be modified to include catalyzed plasma bicarbonate formation. Due to high intracellular concentration of carbonic anhydrase, the CO$_2$ hydration/dehydration is enzymatically catalyzed to proceed to about $10^4$ times faster than the uncatalyzed process in plasma. Therefore, intracellular hydration/dehydration reactions can be assumed to be at equilibrium relative to exchanges of CO$_2$; and the interacting influences of bicarbonate, hydrogen ions and dissolved CO$_2$ can then be described quantitatively by Equation (2.9a) (Klocke, 1987):
\[ [H^+]_{rbc} = K' f_{water} \frac{[CO_2]_{rbc}}{[HCO_3^-]_{rbc}} \] (2.9a)

where

\([CO_2]_{rbc}\) is the dissolved \(CO_2\) concentration inside the RBC expressed in per unit volume of RBCs.

\([H^+]_{rbc}\) and \([HCO_3^-]_{rbc}\) are the concentrations of intracellular hydrogen and bicarbonate ions, respectively; and they are expressed in per unit volume of RBCs.

\(K'\) is the apparent first dissociation constant of carbonic acid, and it includes a factor which allows for the substitution of total dissolved \(CO_2\) concentration for carbonic acid.

\(f_{water}\) is the fraction of water inside the RBC, and it is approximately 0.72 under normal conditions (Meldon, 1984). \(f_{water}\) is introduced here to account for the fact that the concentrations of the involving species are expressed in unit volume of RBC phase instead of unit volume of cell water.

Transforming Equation (2.9a) to the logarithmic form results in the familiar Henderson-Hasselbalch equation

\[ pH_{rbc} = pK' + \log \left( \frac{[HCO_3^-]_{rbc}}{[CO_2]_{rbc}} \right) - \log(f_{water}) \] (2.9b)

where \(pH=-\log([H^+]_{rbc})\) and \(pK'=-\log(K')\). The kinetic and equilibrium constants for hydration and dehydration reactions which were taken from the literature (Gibbson and Edsall, 1963; Garg and Maren, 1972; Nunn, 1987) are summarized in Table 2.2.
<table>
<thead>
<tr>
<th>Reactions</th>
<th>Constants</th>
<th>Water</th>
<th>Buffer Saline</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 °C</td>
<td>37 °C</td>
<td>25 °C</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3$</td>
<td>Forward Rate Constant $k_u \text{ (sec}^{-1}\text{)}$</td>
<td>0.03</td>
<td>0.223</td>
<td>0.0318</td>
</tr>
<tr>
<td></td>
<td>Reverse Rate Constant $k_v \text{ (sec}^{-1}\text{)}$</td>
<td>20.0</td>
<td>70.4</td>
<td>18.5</td>
</tr>
<tr>
<td>$\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$</td>
<td>First acid dissociation constant of $\text{H}_2\text{CO}_3$ $K_1 \text{ (M)}$</td>
<td>1.74x10^{-4}</td>
<td>1.58x10^{-4}</td>
<td>4.06x10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.63x10^{-7}</td>
<td>5.01x10^{-7}</td>
<td>6.92x10^{-7}</td>
</tr>
</tbody>
</table>

*Table 2.2*

Reaction rate equilibrium constants for hydration/dehydration reactions in aqueous media.
2.1.2.b Carbamino Carriage

Amino groups of hemoglobin and plasma proteins have the ability to combine directly with CO₂ (Klocke, 1987):

\[ R-NH_3^+ \Leftrightarrow R-NH_2 + H^+ \]  

(2.Va)

\[ R-NH_2 + CO_2 \Leftrightarrow R-NHCOOH \]  

(2.Vb)

where \( R \) is the protein moiety. In a protein, the amino groups involved in the peptide linkages between amino acid residues cannot combine with CO₂. Carbamino carriage is therefore restricted to the one terminal group (\( \alpha \)-amino) in each protein and to the side chain amino groups (\( \epsilon \)-amino). However, the \( \epsilon \)-amino groups of hemoglobin have a high \( pK_a \) (where \( K_a \) is the acidic dissociation constant of the amino group, see Reaction (2.Va)), \( pK_a=10 \); this precludes any significant contribution to CO₂ binding under physiological circumstances (Klocke, 1987)). It was calculated that the arterial-venous difference of carbamate accounted for 10.5\% (normal DPG concentration) and 27\% (in the absence of DPG) of the CO₂ excreted by the lung despite a relatively low absolute concentration. This accounts for the major part of Haldane effect, which is the difference in the quantity of CO₂ carried, at constant \( P_{CO_2} \), in oxygenated and reduced blood. The remainder of the effect is due to the increased buffering capacity of deoxygenated (reduced) hemoglobin (Nunn, 1987). In other words, the Haldane effect is due to the fact that combination of O₂ with hemoglobin causes hemoglobin to become a stronger acid, and this in turn displaces CO₂ from the blood in two ways. Firstly, the more acidic hemoglobin has less tendency to combine with CO₂, thereby releasing additional CO₂ from carbamino hemoglobin. Secondly, the increased acidity of hemoglobin causes it to release excess of H⁺, and this favors the conversion of more \( HCO_3^- \) into carbonic acid which then dissociates and releases CO₂.
The amount of CO₂ combined with -NH₂ groups of plasma protein to from carbamino compound is so small as to be physiologically negligible. Almost all is carried by hemoglobin, and deoxygenated hemoglobin is about 3.5 times as effective as oxyhemoglobin. The actual $P_{CO₂}$ has very little effect upon the quantity of CO₂ carried in this manner, throughout the physiological range of $P_{CO₂}$ (Nunn, 1987). Formation of carbamino compounds does not require the dissolved CO₂ to be hydrated and so is independent of carbonic anhydrase. The reaction would be of particular importance in a patient who had received a carbonic anhydrase inhibitor. The arterial/venous difference in carbamino carriage had been reported to be lost in certain regions of the body when a patient inhales 100% O₂ at a pressure of about 3 atmospheres absolute, since the O₂ dissolved in the arterial blood is then sufficient for metabolic requirements and very little reduced hemoglobin appears in the venous blood (Nunn, 1987). It was suggested that the loss of the arterial/venous difference in carbamino carriage of CO₂ under these conditions resulted in tissue retention of CO₂, and was a major factor in the cerebral toxic effect produced by high O₂ tension (Klocke, 1987).

The competition effect between CO₂ and DPG binding to hemoglobin is known to be resulting from the fact that CO₂ combines with the α-amino groups of the α- and β-chains of hemoglobin and DPG interacts with the α-amino group of the β chain. The mechanism of the antagonism between CO₂ and DPG binding to hemoglobin was established by Bunn and Briehl (1970). Such an effect was first demonstrated by showing that substantial decrease in alkaline ($pH$) Bohr effect (measured as $ΔlogP_{50}/ΔpH$) caused by CO₂ was abolished by stoichiometric amounts of DPG. However, O₂ affinity of hemoglobin in the presence of DPG was still considerably decreased by CO₂, showing that deoxyhemoglobin still combined with more CO₂ than oxyhemoglobin. The explanation for these results was that DPG was displacing the CO₂ from the α-amino group of the β-chain and that the increase in the $pH$ Bohr effect
caused by DPG described earlier exactly balances the decrease caused by the combination of the \(\alpha\)-amino group of the \(\alpha\)-chain.

It has been shown that in the absence of organic phosphates (DPG and ATP) the binding curve of \(\text{CO}_2\) to human deoxyhemoglobin cannot be fitted to single values of \(K_c\) (\(K_c\) is the \(\text{pH}\)-independent association constant for carbamino \(\text{CO}_2\) binding to hemoglobin) and \(K_z\) (\(K_z\) is the ionization constant for Hb-NH\(_3^+\), the protonated form of the amino groups of hemoglobin that are able to form carbamino \(\text{CO}_2\)) (Kilmartin and Rossi-Bernardi, 1973).

\[
\text{Hb-NH}_3^+ \leftrightarrow \text{Hb-NH}_2 + H^+; \quad K_z = \frac{[\text{Hb-NH}_2][H^+]}{[\text{Hb-NH}_3^+]} \quad (2.V1a)
\]

\[
\text{CO}_2 + \text{Hb-NH}_2 \leftrightarrow \text{Hb-NHCOO}^- + H^+; \quad K_c = \frac{[\text{Hb-NHCOO}^-][H^+]}{[\text{Hb-NH}_2]} \quad (2.V1b)
\]

This suggested that \(\alpha\)- and \(\beta\)-chains have a different reactivity toward \(\text{CO}_2\). A good fit to the \(\text{CO}_2\)-binding data was obtained if two \(\text{pH}\)-dependent association constants for \(\text{CO}_2\) binding are assumed, a high affinity (\(\lambda'\)=650 M\(^{-1}\)) and a low affinity constant (\(\lambda''\)=240 M\(^{-1}\)). On addition of DPG or ATP, the carbamino \(\text{CO}_2\) binding to human deoxyhemoglobin at 37 °C is decreased by about 30\% at \(P_{\text{CO}_2}\) of 40 mmHg. As mentioned previously, although ATP exerts effects on hemoglobin similar to those of DPG, it appears to be largely chelated with Mg\(^{2+}\) and Ca\(^{2+}\) inside the RBC, so that the "free" ATP concentration is low and that DPG is the more important allosteric factor of these two organic phosphates. Experimentally, it was observed that the carbamino \(\text{CO}_2\) binding curve with DPG or ATP coincided exactly with the binding curve for the higher affinity binding site (\(\lambda'\)) (Kilmartin and Rossi-Bernardi, 1973). Their interpretation for this finding was that DPG or ATP completely displaced \(\text{CO}_2\) from the low-affinity site (\(\lambda''\)); thus this site must be the \(\beta\)-chain \(\alpha\)-amino group because it was clearly involved in
DPG binding to deoxyhemoglobin. The higher affinity CO\textsubscript{2} binding site (\(\lambda'\)) is therefore the \(\alpha\)-chain \(\alpha\)-amino group. The preceding simplified structural interpretation allowed Kilmartin and Rossi-Bernardi (1973) to derive the equations for competitive interaction between DPG and CO\textsubscript{2}. The total carbamino CO\textsubscript{2} bound by the \(\alpha\)- and \(\beta\)-chains of hemoglobin in the presence of DPG is

\[
\phi = \frac{2 \lambda_\alpha [CO_2]}{1 + \lambda_\alpha [CO_2]} + \frac{2 \lambda_\beta [CO_2]}{1 + \lambda_\beta [CO_2] + K_{DPG} [DPG]}
\]

(2.10)

where

\(\phi\) is the moles of CO\textsubscript{2} bound per mole hemoglobin tetramer.

\(\lambda_\alpha\) (or \(\lambda'\)) and \(\lambda_\beta\) (or \(\lambda''\)) are the pH-dependent association constants for CO\textsubscript{2} binding for the \(\alpha\)-amino groups of the \(\alpha\)-chain and \(\beta\)-chain, respectively.

\(K_{DPG}\) is the pH-dependent association constant of DPG and hemoglobin.

It is worth noting that \(\lambda_\beta\) and \(K_{DPG}\) are the pH-dependent association constants; their pH dependence in the physiological range operates in the opposite directions. \(K_{DPG}\) increases with decreasing pH, leading to a higher affinity for DPG, whereas \(\lambda_\beta\) decreases since the fraction of charged \(\beta\)-chain \(\alpha\)-amino groups able to combine with CO\textsubscript{2} decreases.

It should be emphasized that these equations provide only a simplified treatment of interaction between CO\textsubscript{2}, DPG and hemoglobin. In particular the problem of a hemoglobin tetramer with one CO\textsubscript{2} molecule bound has not been considered because it was not clear whether this would totally exclude DPG binding or merely reduce it. The assumption made by Kilmartin and Rossi-Bernardi, that two CO\textsubscript{2} molecules bound per tetramer would completely exclude DPG binding, seems reasonable on stereochemical grounds. In the physiological pH range, \(\phi\) will vary between 0 and 4. Equation (2.10)
was used to fit CO₂-binding to human deoxyhemoglobin with values of \( \lambda_\alpha = 650 \text{ M}^{-1} \) and \( \lambda_\beta = 240 \text{ M}^{-1} \) at pH 7.4 and 37 °C (Kilmartin and Rossi-Bernardi, 1973). While Garby and de Verdier (1971) and Kilmartin and Rossi-Bernardi (1973) calculated the association rate constant for DPG binding to deoxyhemoglobin to be in the range of \( 5 \times 10^2 \text{ M}^{-1} \) and \( 1.5 \times 10^3 \text{ M}^{-1} \).

Ferguson (1936) examined the effect of oxygen saturation at various level on the carbamino carriage of CO₂, and found that the difference in bound carbamino CO₂ between oxy- and deoxyhemoglobin is linear with oxygen saturation (data is given in *Applied Respiratory Physiology* by Nunn, 1987).

2.1.2.c Anion Transporter

In the tissue \( P_{CO2} \) is 46 mmHg, while the \( P_{CO2} \) of the arterial blood entering the tissue capillary is 40 mmHg. Although the pressure difference is small, CO₂ easily diffuses from the tissue to the blood. The diffusion process is governed both by a high lipid solubility of CO₂ and by a short diffusion distance. As CO₂ enters the blood, it dissolves physically in the extracellular water phases, forms carbamino compounds predominantly by binding to intracellular hemoglobin; and most of CO₂ is hydrated to carbonic acid, which subsequently dissociates into bicarbonate and hydrogen ions. The extracellular spontaneous hydration of CO₂ is too slow a process to provide any significant contribution. Therefore, the bicarbonate production takes place almost entirely within the RBC. The intracellular bicarbonate formation is accompanied by a production of hydrogen ions, which are buffered to a greater extent, mainly by hemoglobin. The removal of H⁺ is very efficient because the buffer properties of hemoglobin are amplified by the \( \text{O}_2 \)-linked \( pK \) shifts that accompany the transition from oxyhemoglobin to deoxyhemoglobin, the Haldane effect. Unless bicarbonate ions are also removed from the intracellular phase, the formation of bicarbonate ions will stop when the bicarbonate concentration is in equilibration with \( P_{CO2} \) and \( pH \) according to the
Henderson-Hasselbalch equation. In fact, intracellular bicarbonate is removed by the so-called chloride-bicarbonate exchange (also known as the "Hamburger shift" or the "chloride shift") across the RBC membrane, whereby 75% of the formed bicarbonate is stored in the plasma until the blood reaches the lung capillary, where the reactions would run backward in this so-called "Jacobs-Stewart cycle" (Weith et al., 1982; Frohlich and Gunn, 1986). Most of the CO₂ liberated in the lung capillaries is generated from the plasma HCO₃⁻. As intracellular HCO₃⁻ decreases, plasma HCO₃⁻ enters the RBC in exchange for Cl⁻ and the production of free CO₂ through the action of carbonic anhydrase. Although the contribution of plasma to the total CO₂ carriage is greater than that of the RBC, almost all CO₂ excreted from the plasma must first enter the RBCs so that it can be processed to a form (molecular CO₂) that is readily excreted.

HCO₃⁻/Cl⁻ exchange across the RBC membrane plays a major determinant role in the CO₂ exchange and transport for the reason mentioned above that under normal physiological conditions 80% of CO₂ transfer/exchange is derived from the hydration of plasma and RBC bicarbonate. In addition, this pathway provides a crucial link between two compartments with different H⁺ buffering characteristics. It has been shown that a specialized transport system, involving a major RBC membrane protein (band 3), is involved in the electroneutral translocation of anions into and out of RBCs. It is designated as the band 3 protein because it can be easily localized on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophograms, where it is found in the third major band from the top. The exchange of these anions by band 3 is essential to the respiration of CO₂; as a result, there exists an extensive amount of literature on the band 3 anion transport system. There are various reviews on band 3 biochemistry, exchange kinetics and anion transport inhibitors (Cabantchik et al., 1978; Lowe and Lambert, 1983; Jennings, 1985; Frohlich and Gunn, 1986; Brahm, 1986; Passow, 1986; Jennings, 1989). Some investigators have reported that the largest of the resistances
involved in CO₂ transport is associated with anion exchange (Weith and Brahms, 1980; Crandall and Bidani, 1981). For instance, Crandall et al.'s (1981) study in isolated rat lungs suggested that CO₂ transfer in capillary beds is adversely affected in vivo when the rate of RBC HCO₃⁻/Cl⁻ exchange is abnormally low. While others have reported that this exchange is not rate limiting in CO₂ transport under resting conditions; however, this exchange becomes a bottleneck for CO₂ transport under exercising conditions due to the significantly reduced transit time through the capillary (Weith et al., 1982). Therefore, it is important that we are able to describe this transmembrane exchange process.

Hyperventilation under resting conditions appears when the elimination of CO₂ from the tissue is impeded. Possible conditions causing a reduced CO₂ elimination are restricted diffusion of CO₂ in pulmonary diseases accompanied by hypercapnia, inhibition of intracellular carbonic anhydrase, and an impaired anion transport due to a reduced number of transport sites per RBC and/or inhibition of Jacobs-Stewart cycle (Weith et al., 1982). Since carbonic anhydrase activity greatly exceeds the needs of the organism, a pronounced reduction of the enzyme activity may have no pathophysiological consequences. In contrast, a slight reduction of anion exchange capacity causes an immediate compensatory hyperventilation which seems to indicate that anion transport system has practically no reserve capacity at all. Crandall et al. (1981) conducted experiments to confirm the hypothesis that inhibition of RBC anion exchange can significantly affect both CO₂ excretion and postcapillary pH equilibration. Furthermore, because a number of commonly used drugs, such as salicylate, furosemide and anesthetics are known to inhibit the rate of RBC anion exchange, these results thus imply a potential clinically significant effect on CO₂ transfer in vivo by inhibition of the anion exchange kinetics, especially in patients with limited cardiopulmonary reserve (Crandall et al., 1982; Weith and Brahms, 1985). In order to
study the respiratory function of blood, it is important to develop a mathematical expression for describing this anion exchange across the RBC membrane. The development of the flux equation as a function of two kinetic parameters and the intra- and extracellular anion compositions is discussed in Chapter 5.

2.1.2.d  **Buffers in Blood**

CO₂ produced by aerobic metabolism is transported in such a way that excess H⁺s are produced, both through dissociation of carbonic acid and the formation of carboxamino compounds. Anaerobic metabolism produces lactic acid, a further source of H⁺s. The total amount of H⁺ produced daily in man in the form of nonvolatile acids is 50-70 mEq. Accordingly, blood must possess potent buffering system to prevent large changes in acidity associated with CO₂ transport. In the blood, proteins - for example, the plasma proteins - can serve as buffers because both their free carboxyl and their free amino groups dissociate.

\[
RCOOH \leftrightarrow RCOO^- + H^+ \quad (2.VIIa)
\]

\[
RNH_3^+ \leftrightarrow RNH_2 + H^+ \quad (2.VIIb)
\]

A more important buffer system is produced by the dissociation of the imidazole groups of the histidine residues in hemoglobin.

\[
\begin{align*}
\text{HN} & \quad \text{C} \quad \text{NH}^+ \\
\text{HC} & \quad \text{C} \quad \text{R} \\
& \quad \leftrightarrow \\
\text{HN} & \quad \text{C} \quad \text{N} \\
\text{HC} & \quad \text{C} \quad \text{R} \\
& \quad + \quad H^+ \quad (2.VIII)
\end{align*}
\]

In the pH 7.0 - 7.7 range, the free carboxyl and amino groups of hemoglobin contribute relatively little to its buffering capacity. However, the hemoglobin molecule contains 38 histidine residues and it is present in large amounts. Thus, the hemoglobin in blood has
six times the buffering capacity of the plasma proteins. In addition, the action of hemoglobin is unique because the imidazole groups of deoxygenated hemoglobin dissociate less than those of oxyhemoglobin, making deoxygenated hemoglobin a weaker acid and therefore a better buffer than oxygenated hemoglobin.

Another major buffer system in the blood is the carbonic acid-bicarbonate system because the $H_2CO_3$ level is in equilibrium with dissolved CO$_2$, and the amount of dissolved CO$_2$ is controlled by respiration.

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

(2.1X)

Although blood contains other buffers, such as phosphate, they are not particularly important for short term pH regulation. The system $H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$ has a $pK$ of 6.8. In human plasma, the phosphate concentration is too low (e.g., $[HPO_4^{2-}]_{pl}$ and $[H_2PO_4^-]_{pl}$ are about 2 mM as compare to $[HCO_3^-]_{pl}$ of about 30 mM) for this system to be a quantitatively important buffer.

As illustrated in Figure 2.1, generation of free CO$_2$ via the carbamate and dehydration reactions consume H$^+$. Conversion of HCO$_3^-$ to CO$_2$ requires an equal number of protons. Release of CO$_2$ from carbamate utilizes even more H$^+$. The formation of carbamic acid from the carbamate ion consumes a single proton. As CO$_2$ is released from the carbamic acid, the concentration of uncharged amino groups increases. A portion of these groups ionizes, consuming more H$^+$. Generation of CO$_2$ by carbamate pathway requires more than a single proton, the exact number depending on the values of $K_z$ and $K_c$ and their change with oxygenation. The number has been estimated to vary between 1.2 and 1.8 protons from $pH$ 6.9 to 7.9 in human blood. Also it appears to be a function of $O_2$ saturation (Klocke, 1973). Hemoglobin, which is the principal source of protons for both reactions, provides H$^+$ by two mechanisms.
First, the intracellular contents are potent buffers, releasing about 3 moles of proton per mole of hemoglobin monomer with a unit pH change (Sigggaard-Anderson, 1971). Second, the pK values of certain acidic groups on the hemoglobin molecule change with oxygenation, releasing Bohr protons (Perutz, 1978). The number of H⁺s released with binding of a molecule of O₂ can be computed from the relationship between two linked functions (Equation 2.1), as described by Wyman (1964). As an approximation, about 0.5-0.7 H⁺ is released with binding of one O₂ molecule under normal circumstances, but this ratio varies significantly, depending on pH, PCO₂, 2,3-DPG concentration, and even the O₂ saturation of blood (Hlastala and Woodson, 1975).

Consequently, for the opposite event of CO₂ uptake and O₂ release in the tissue capillaries, the hydration reaction, the carbamino reactions, the dissociation of the positively charged amino groups, and the deoxygenation of hemoglobin all produce H⁺s. However, as a result of the various buffer systems mentioned above, the actual increase in H⁺ concentration is considerably less than that indicated by these reactions. The buffering action can be expressed through the following equation

\[ \frac{d[H^+]}{dt} = \frac{d[H^+]}{d(pH)} \frac{d(pH)}{d[H^+]_{app}} \frac{d[H^+]_{app}}{dt} = \frac{2.303 [H^+]_{app}}{d[H^+]_{app}} \]

where

\[ d[H^+]_{app}/dt \] is the actual rate of increase in H⁺ concentration.

\[ d[H^+]_{app}/dt \] is the apparent rate of increase in H⁺ concentration.

\[ -d[H^+]_{app}/d(pH) \] is the buffering capacity of the medium, \( \beta \). \( \beta \) is the amount of base added or acid removed per pH unit change and it can be determined experimentally.

In addition, because pH=-log[H⁺] and \( d[H^+]_{app}/d(pH) = -2.303[H^+] \), \( d[H^+]_{app}/dt \) can then be rewritten as \( (2.303[H^+]_{app}/dt)/\beta \).
Buffering capacity can be determined in two ways. First, all the detailed reaction schemes can be combined with a statement of electroneutrality to include the ionic equilibria reactions to form a complete, but cumbersome, set of equations which describe the exact interactions (Butler, 1964). This approach is, however, primarily useful in model systems that contain one or at most two, buffers for which the equilibrium constants and exact concentrations are known. In blood, this type of development is generally not applicable due to the complex mixture of buffers. The second approach which describes buffer action has found more widespread application to describe mixtures of buffers. It relies on the definition of buffers as “substances which by their presence in solution increase the amount of acid or alkali that must be added to cause a unit change in pH”. A good buffer has a large value of $\beta$. This value is always positive, so that the addition of base increases the $pH$ while the addition of acid decreases the $pH$. $\beta$ is normally determined experimentally, and is, in general, a function of $pH$.

For very simple systems, $\beta$ can be determined from chemical equilibrium, the first method described above. In mixtures of buffers, $\beta$ can be determined experimentally by titration of solution of interest. The usefulness of this approach when applied to plasma and hemoglobin solutions at physiological $pH$ is twofold. First their titration curves, $d[H^+]_{app}$ vs. $d(pH)$, are effectively linear. Second, when dissolved CO$_2$ levels are varied, the differential change in bicarbonate ion is equal to the non-bicarbonate buffer value as (Klocke, 1987):

$$- \frac{d[HCO_3^-]_{pl}}{d(pH)_{pl}} = \beta_{pl}$$  \hspace{1cm} (2.12a)

$$- \frac{d[HCO_3^-]_{rbc}}{d(pH)_{rbc}} = \beta_{rbc}$$  \hspace{1cm} (2.12b)
where $\beta_{rbc}$ and $\beta_{pl}$ are the intracellular and extracellular buffering factors, respectively. Therefore, the non-HCO$_3^-$ buffering power of physiological solutions is expressed in $\{d[HCO_3^-]/d(pH)\}$ with HCO$_3^-$ concentration expressed as millimoles per liter. Addition of buffers to a solution improves buffering capacity and thus the increment in HCO$_3^-$ concentration with any given change in pH. The buffer value for plasma varies with the total proteins and phosphates present. For hemoglobin, production of H$^+$s via carbamate and oxyhemoglobin formation can be regarded as a change in the buffer value from some standard condition.

Plasma separated from RBCs and then equilibrated with CO$_2$ has the ability to absorb CO$_2$ because the buffering provided by plasma proteins. The capacity of "separated" plasma is proportional to protein concentration. However, this is substantially less than the apparent buffering power of "true" plasma - plasma that has been separated from RBCs after equilibration with CO$_2$. Plasma CO$_2$ content then reflects not only plasma buffering but also buffering provided by intracellular hemoglobin. HCO$_3^-$ formed due to buffering of CO$_2$ inside the cell exchanges for plasma Cl$^-$, thereby increasing plasma HCO$_3^-$ more than could be achieved by plasma buffering alone. Erythrocyte buffering power, corrected for the intracellular water content, is greater than that of plasma. Whole blood, a mixture of plasma and RBCs, has an intermediate value. The buffering powers of "true" plasma, erythrocytes, and whole blood are predominantly a function of hemoglobin concentration; and these buffering powers all pertain to solutions equilibrated in vitro.
2.2 Fluid Mechanics of Blood Flowing in Tubes and Enhancement of Gas Transport Due to Migrations of RBCs

The development of a mathematical model of mass transport in microvessel requires knowledge not only of the basic mechanism of transport and the complex chemical and physical interactions but also the hydrodynamics of the system. In this section, the rheology of red cell suspensions is described. Emphasis is given to the rheology of blood in tube flow and its possible relationship to gas transport which is the main goal of this work. The rheological properties of blood have been studied in detail using coaxial cylinder and capillary viscometers. Since these viscometers are large-scale devices, the measurements can be applied to a homogeneous fluid theory. The viscosity of blood has been found to be non-Newtonian at very low shear rates, presumably due to breakup of RBC clusters. In a strongly sheared system (shear rate > 100 sec$^{-1}$) the viscosity of blood approaches a constant, “Newtonian” value. Plasma always exhibits Newtonian behavior. The specification of the velocity field is simplified considerably if the non-Newtonian behavior of blood is neglected. The approximation is reasonably acceptable for a system operating in a high shear rate region.

2.2.1 Distribution of Cells and Velocity Profiles of Flowing Blood

In large microvessels, many red cells are intersected by a cross section of the vessel; the flow is then complicated by the interactions between cells. Observations in vivo as well as in vitro show that there is a thin layer near the wall which has fewer red blood cells in it than average. This is due partly to wall exclusion and also lateral migration of cells which leads to a radial distribution of hematocrit with a higher hematocrit at the center and lower near the wall. Another feature is that velocity is blunted in the central portion of the tube compared to Poiseuille flow (Figure 2.4).
**Figure 2.4:** RBC and plasma velocity profiles and hematocrit profile of RBC suspensions flowing in microvessels.

Blunting of the flow profile is a function of hematocrit, flow rate and microvessel diameter; profiles were found to be blunt at high hematocrits and low flowrates, and/or in small microvessels. With increasing diameter, the profile approaches a parabolic form, such that in vessels above 75 μm in diameter the deviation from a parabolic profile is below 5% under *in vitro* conditions (Bugliarello and Sevilla, 1970; Lee *et al.*, 1983). However, it should be mentioned that more blunted profiles were reported from *in vivo* measurements in arterioles and venules (Pitman and Ellsworth, 1986). The central region travels at higher velocity than the average for the suspension as a whole. This together with the existence of the cell-depleted layer near the vessel wall cause the average hematocrit in the tube, $H_T$, to be generally less than that in the reservoir to which the blood is discharged to, $H_D$, under steady-state conditions. This phenomenon of $H_T/H_D$ being less than one in vessels with diameter smaller than about 500 μm is generally referred to as the Fahraeus effect (Cokelet, 1987).

Several flow models which take into account some or all of above mentioned flow characteristics have been proposed by different investigators with varying degrees of success. Most of the models assume a two layered flow consisting of a cell-depleted
wall layer surrounding a core of suspension. The hematocrit and flow relationships used in this work are the same as those which were used by Nair et al. (1989). The description of RBC distribution in the vessel used by Nair et al. (1989) is of the form suggested by Lih (1969), with a modification to exclude the cell-free region. The velocity profile of RBC suspension in microvessel was treated as parabolic with a slight blunting in the center to account for the deviation from the Poiseuillean profile.

2.2.1.a **Hematocrit Profile**

From observations of hematocrit profiles (Caro et al., 1978; Goldsmith and Turitto, 1986), it has been found that the radial hematocrit distribution can be expressed as:

\[
h(r) = h_m \left[1 - \left(\frac{r}{r_r}\right)^m\right] \tag{2.13a}
\]

\[
h(r) = 0 \tag{2.13b}
\]

\[
\left(h_m \geq 0, m' \geq 0\right)
\]

where

- \(r\) is the radial coordinate.
- \(h(r)\) is the hematocrit profile, the volume fraction of RBCs at any radius \(r\).
- \(r_r\) is the radius of the cell-rich region, the difference of tube radius and the thickness of the cell-free layer.
- \(r_c\) is the radius of the microvessel.
- \(h_m\) and \(m'\) are constants which need to be determined.

The cell-free layer adjacent to the capillary wall, \(\delta\), was taken as that due to geometric consideration alone (see Figure 2.4):

\[
\delta = r_c + t_{rbc} - \sqrt{r_c^2 - r_{rbc}^2} \tag{2.14}
\]
where

\( r_{rbc} \) is the radius of the RBC disc.

\( t_{rbc} \) is the maximum half thickness of the RBC disc.

2.2.1.b Velocity Profiles

Different velocity profiles are used for the plasma and the RBCs (Figure 2.4). The profiles are parabolic with a slight blunting, and differ by a constant - the slip.

\[
V_{pl}(r) = A \left[ 1 - \left( \frac{r}{r_c} \right)^2 \right] \quad r_r \leq r \leq r_c \quad (2.15a)
\]

\[
V_{pl}(r) = D \left[ 1 - B \left( \frac{r}{r_c} \right)^2 \right] \quad 0 \leq r \leq r_r \quad (2.15b)
\]

\[
V_{rbc}(r) = D (1 - slp) \left[ 1 - B \left( \frac{r}{r_c} \right)^2 \right] \quad 0 \leq r \leq r_c \quad (2.15c)
\]

\[(0 \leq B \leq 1) \text{ and } (0 \leq slp \leq 1)\]

where

\( B \) is the blunting factor based on Pittman and Ellsworth's work (1986).

\( V_{pl}(r) \) and \( V'_{pl}(r) \) are the plasma velocities at radius \( r \) in the cell-free and cell-rich region, respectively.

\( slp \) is a "slip" constant based on Sinha's work (1936). It is the difference in the velocity of the suspending fluid and the particle at a particular point due to the finite size of the particle, used as 0.1 in all cases.

\( V_{rbc}(r) \) is the RBC velocity at radius \( r \) in the cell-rich region.

\( A \) and \( D \) are constants corresponding to centerline velocities which need to be determined for each case.

Because RBCs are semi-solid entities suspending in the plasma, the properties such as velocities and hematocrit actually vary with position in a discontinuous fashion.
However, as a simplification, these properties are treated here as varying continuously. So, for example, the RBC velocity at a given radial position is an average velocity averaging over time, even through at any given instant of time that particular position may or may not be occupied by a RBC. The parameters $A$, $D$, $h_m$, and $m$ can be obtained by simultaneous solution of equations which describe: the continuity of the plasma velocity profile, the overall RBC mass balance, the overall plasma mass balance and the definition of tube hematocrit (see Section 6.1.5.d).

2.2.2 Shear-induced Augmentation of Gas Transport in Blood

The presence of particles is expected to increase the rate of transverse mass transport in flowing suspensions over the rate in the suspending medium alone. It is postulated that the augmentation in transport rates is due to lateral fluid movements caused by particle rotations and collisions. Prediction of mass transfer rates is generally more complicated for flowing suspensions than for simple Newtonian fluids. However, mass transfer equations and correlations for Newtonian fluids might work well for suspensions provided the proper “effective” diffusivity is used. In reality, the transverse mass flux vector is consisted of two parts, effective diffusion under stationary flow condition and convection due to the lateral fluid movements; consequently, the “effective” diffusivity lumps these two terms together.

Leal (1973) and Nir and Acrivos (1976) considered the cases of dilute suspensions of deformable droplets in the limit of low and high Peclet numbers, respectively; both suggested a linear dependence of enhanced diffusivity on particle concentration, $H_D$. Increases in $H_D$ should causes increases in transport rates; until particle crowding begins to restrict the lateral excursions of the particles. At very high particle concentrations ($H_D=0.99$), dispersive particle migration of particle is reduced, suggesting that augmentation is zero. Using these type of augments, Zydeney and Colton
(1988) have suggested the following empirical correlation for this enhanced solute \( (O_2) \) transport:

\[
D_{rbc} = a^2 \gamma \zeta H_D(1 - H_D)^\sigma
\]  

(2.16)

where

- \( D_{rbc} \) is the shear-induced diffusion coefficient of red blood cells.
- \( a \) is the particle radius of red cell.
- \( \gamma \) is the local shear rate.
- \( \zeta \) and \( \sigma \) are empirically fitted parameters.
- \( H_D \) is the volume fraction of the suspended red blood cells or hematocrit.

For deformable particles (liquid drops and red cells) of characteristics radius \( a \) in a local shear rate \( \gamma \), the numerous data on particle and saturated red cell diffusion were empirically fitted to give values for parameters \( \zeta \) and \( \sigma \), and the best fit values are \( \zeta=0.15 \) and \( \sigma=0.8 \). The excellent agreement between the above correlation and the experimental data suggested that their concept of augmented solute transport arising primarily from shear-induced particle and fluid migration and not from particle rotation is reasonable, at least for particle volume fractions which are either very low nor extremely high. This empirical model can be used to quantify the augmentation of oxygen transport observed under condition in which hemoglobin is not actively involved in the transfer process.

Since oxygen is largely transported by attachment to the hemoglobin of red cells, diffusion of red cells can produce an additional increased rate of mass transfer. Lateral migrations of red cells move oxygen-saturated red cells to unsaturated region where oxyhemoglobin could serve as a strong source for release of oxygen; conversely, unsaturated hemoglobin could serve as a strong sink for oxygen uptake on subsequent radial migration into an oxygen-saturated region. In an attempt to incorporate the
amplifying effect due to the oxygen storage potential of the hemoglobin into the picture, the red cells are treated as flat disks with their faces parallel to the wall and occasionally undergo flip-flop end over end. Consequently, one-dimensional oxygen transfer from plane sheets of hemoglobin is assumed. The total oxygen flux through the suspension can be expressed as follows (Zydney and Colton, 1988):

\[
Total \, O_2 \, flux = -D_{O_2, SF} \frac{\partial}{\partial r} [h(r) [O_2]_{rbc} + (1 - h(r)) [O_2]_{pl}] \\
- D_{O_2, rbc}^S \frac{\partial}{\partial r} [h(r) ([O_2]_{rbc} + [HbO_2])] - D_{O_2, pl}^S \frac{\partial}{\partial r} [(1 - h(r)) [O_2]_{pl}]
\]

where

- \( r \) is the direction which is parallel to the velocity gradient.
- \( D_{O_2, SF} \) is the effective diffusivity of oxygen under unshared condition.
- \( D_{O_2, rbc}^S \) and \( D_{O_2, pl}^S \) are the shear-induced oxygen diffusivities in the red cell and plasma phases, respectively.
- \([O_2]_{rbc}\) and \([O_2]_{pl}\) are the dissolved oxygen concentrations inside the RBC and in the plasma, respectively.
- \([HbO_2]\) is the concentration of oxygen that is bound as oxyhemoglobin.

If particle rotations make a negligible contribution to the overall oxygen flux, then \( D_{O_2, rbc}^S = D_{O_2, pl}^S = D_{rbc} \); and \( D_{rbc} \) is given by Equation (2.16). If the gradient in RBC volume fraction is negligible, then \( h(r) \) is a constant which is independent of position and is equal to \( H_D \). In addition, if chemical reaction of oxygen and hemoglobin takes place rapidly by comparison to diffusion, it may be assumed that the free and bound oxygen are in instantaneous equilibrium and that the two concentrations are related through the ODC. Furthermore, if the solubility coefficients of oxygen in the red cell and the plasma are assumed approximately equal, then the total oxygen flux may be written as
Total \( O_2 \) flux = \(- D_{O2, SF} \frac{\partial [O_2]}{\partial r} - D_{rbc} \frac{\partial [O_2]}{\partial r} - H_D D_{rbc} C_{heme,rbc} \left( \frac{dS}{d[P_{O2}]} \right) \frac{\partial [O_2]}{\partial r} \) 

With the driving force taken to be the concentration of the total dissolved oxygen in the suspension, \([O_2]\), the effective diffusion coefficient becomes

\[ D_{O2, eff} = D_{O2, SF} + D_{rbc} (1 + m) \] (2.19a)

and \( m = \frac{H_D C_{heme,rbc}}{\alpha_{O2}} \frac{dS}{dP_{O2}} \) (2.19b)

where

\( D_{O2, eff} \) is the effective diffusivity of oxygen under shared condition.

\( C_{heme,rbc} \) is the heme concentration inside the RBC.

\( \alpha_{O2} \) is the Bunsen solubility coefficient of \( O_2 \) in the blood.

\( \frac{dS}{dP_{O2}} \) is the slope of the ODC, and it is a nonlinear function of \( P_{O2} \).

\( m \) is the oxygen carriage potential of hemoglobin calculated based on the local operating slope of the ODC (Equation (2.19b)).

The expression for \( D_{O2, eff} \) derived here is the same as that obtained by Zydney and Colton (1988), except that they had a different interpretation of \( m \). They defined \( m \) as the ratio of bound to free oxygen concentrations and assumed it to be approximately constant.

Zydney and Colton (1988) were not the first to derive an \( O_2 \) augmentation model. In fact, Diller and Mikic (1983) had previously developed a similar model for augmented oxygen transport in blood which was based on the concept that shear-induced motion of red cell can be modeled in an analogous manner to the Brownian motion of molecules. Their results for \( D_{O2, eff} \) can be expressed as
\[ D_{O2,\text{eff}} = D_{O2,\text{SF}} + D_{rbc} \cdot g \cdot (H_D + M) \]  \hspace{1cm} (2.20a)

and

\[ M = \frac{H_D \cdot C_{\text{heme,rbc}}}{\alpha_{O2}} \cdot \left( \frac{S_{I_{P_{O2}=P_{O2,ext}}} - S_{I_{P_{O2}=P_{O2,in}}}}{P_{O2,ext} - P_{O2,in}} \right) \]  \hspace{1cm} (2.20b)

where

- \( P_{O2,\text{ext}} \) is the oxygen tension in the environmental chamber surrounding the membrane film.
- \( P_{O2,\text{in}} \) is the oxygen tension of the inlet red cell suspension.
- \( S_{I_{P_{O2}=P_{O2,ext}}} \) and \( S_{I_{P_{O2}=P_{O2,in}}} \) are the fractional saturations of the hemoglobin molecules under the oxygen partial pressures of \( P_{O2,\text{ext}} \) and \( P_{O2,\text{in}} \), respectively.
- \( g \) is a measure of the departure of the hemoglobin-oxygen binding reaction from equilibrium (0 ≤ g ≤ 1); in the limit of reaction equilibrium, g=1.
- \( M \) is the oxygen carriage potential of hemoglobin calculated based on the overall operating slope of the ODC (Equation (2.20b)).

Equation (2.20a) contains one slight error; namely, the factor \( g \) multiplied by both \( H_D \) and \( M \) terms. This is inappropriate, since nonequilibrium only affects the bound oxygen concentration and not the dissolved oxygen. In the limit of reaction equilibrium, \( g=1 \), Equations (2.20a) and (2.20b) yield an effective diffusivity that is different from that given by Equations (2.19a) and (2.19b). The major difference is that Diller and Mikic replaces \( m \), the local operating slope, with \( M \), the overall operating slope. Another difference is that Equation (2.20a) does not include the term \((1-H_D)D_{rbc}\); this is due to the fact that Diller and Mikic neglected the effect of dispersive fluid migrations in their analysis. For unsaturated blood where \( M \gg 1 \), \( D_{O2,\text{eff}} \) is dominated by dispersive cell motion; and as a result, the contribution due to \((1-H_D)D_{rbc}\) is negligible. However, the dispersive fluid motion could become more important under conditions of high shear rates and low values of overall operating slopes.
CHAPTER 3

REVIEW OF COUPLED OXYGEN AND CARBON DIOXIDE
TRANSPORT MODELS

A great number of mathematical analyses of various aspects of respiratory gas exchange has appeared in the literature. These can be divided into the following categories:

1. Models of exchange between RBC and plasma alone (e.g. Sirs, 1970; Klocke, 1973; Forster and Crandall, 1975; Coin and Olson, 1978; Salathe, 1981; Vandegriff and Olson, 1984b and 1984c; for recent reviews on O₂ and CO₂ transport see Baumann et al., 1987 and Klocke, 1987). These studies aimed specifically at a determination of the rate-limiting steps in O₂/CO₂ exchange between two phases of the blood, and have contributed to our knowledge of RBC membrane permeability to gases and exchange of anions, as well as the kinetics of diffusion and chemical reaction within the red cell.

2. Models of exchange of O₂ and CO₂ between blood and the alveoli (Forster, 1957; Wagner and West, 1972; Hill et al., 1973; Hlastala, 1973; Bidani et al., 1978; Singh et al., 1980; Sharan et al., 1987; for a recent review on CO₂ exchange in the lung, see Bidani and Crandall, 1988). These analyses considered the Bohr and Haldane effects, and the implications of a finite diffusional resistance in the lung membrane.

3. Analysis of O₂ transport to tissue (Reneau et al., 1967; McCracken et al., 1972; Meldon and Garby, 1975; Artigue, 1980; Baxley and Hellums, 1983; Federspiel and Popel, 1986; Nair, 1988; Ellsworth et al., 1988; Weerappuli and Popel, 1989; for a recent review on the subject, see Popel, 1989). Consideration was given to the diffusion of O₂ in up to three dimensions, as well as in tissue surrounding the capillary networks. Metabolic O₂ consumption has been modeled by either a zero order, first order, or
Michaelis-Menten kinetic expression. Through the application of complex mathematical analysis, a firmer theoretical base has been given to speculations regarding the distribution of O$_2$ in tissue, and also in regard to vessel orientation and flow directions within the capillary clusters. Most of the models, however, involved a simplified representation of blood chemistry.

4. Calculations of the rate of gas exchange between flowing blood and gas phase in membrane oxygenators (diameters of several hundred $\mu$m) or artificial membrane microvessels (diameters less than 100 $\mu$m). While these have been directed mostly towards the design of artificial lungs, and not in vivo transport, much of the physics and chemistry is the same as have been applied to the analyses of physiological processes. Therefore, these models have utility in providing guidance in designing oxygenators and aiding in the interpretation of membrane lung function, but also in providing guidance in the understanding of gas transport in arterioles and venules of the microcirculation.

A more detailed review on the subject matter of category 4 will be present here because the subject is closely related to the objective of this thesis which is to model the gas transport by flowing blood in microvessels.

3.1 Exchange between Red Blood Cells and Plasma alone
and Transport of Gas to and from Blood Flowing in Capillaries

Under equilibrium conditions, the total amount of CO$_2$ held by the blood may be calculated from the CO$_2$ dissociation curve of total CO$_2$ content versus dissolved CO$_2$ (measured as $P_{CO2}$) which depends on pH and O$_2$ saturation; as in the case of O$_2$, the dissociation curve may be measured experimentally. However, during CO$_2$ exchange the reaction rates and transport effects may be important. Although the rate of each individual processes (as illustrated in Figure 2.1) had been measured separately in vitro,
these processes occur simultaneously during blood $O_2/CO_2$ exchange. To obtain an estimate of the rate of overall gas exchange, or the importance of each step in limiting its rate, these measurements must be considered in a model system, including all different chemical reactions and diffusion elements. A number of investigations have been undertaken to determine the specific influence of the interrelated reaction rates and transport mechanisms on gas transport in the lungs and tissues both experimentally and mathematically. Only a sampling of the literature is quoted here in this chapter; excellent summaries of the results from previous research are included in *Biophysics and Physiology of Carbon Dioxide* (Bauer et al., 1980), Blood Oxygen Transport (Baumann et al., 1987) and Carbon Dioxide Transport (Klocke, 1987) in *Handbook of Physiology*, and in Popel’s review paper on Theory of Oxygen Transport to Tissue (Popel, 1989) in *Critical Reviews in Biomedical Engineering*.

Roughton (1935) in his classical review of CO$_2$ transport should be given credit for pointing out the slow $pH$ reaction and suggesting that uncatalyzed hydration of CO$_2$ in the plasma ultimately would limit the exchange of CO$_2$. This idea was extended by Sirs (1970) whose approximate calculation indicated that while carbonic anhydrase accelerates the equilibration between $[CO_2]_{rbc}$, $[H^+]_{rbc}$ and $[HCO_3^-]_{rbc}$ inside the RBC, it has no effect on the speed of these processes in the plasma, so that the necessary alkalinization of this phase takes place by the uncatalyzed dehydration of HCO$_3^-$. The exchanges of Cl$^-$ and HCO$_3^-$ across the RBC membrane have a negligible effect on plasma $pH$. This uncatalyzed reaction requires several tens of seconds to complete and does not have time to occur in the lung capillaries, but must continue in the blood as it travels through the pulmonary veins and into the arterial tree.

The rates at which blood $O_2$ and CO$_2$ equilibrate with alveolar gas depend on the rates of the various chemical reaction events as well as the pulmonary diffusing capacity and affinity of the blood for $O_2$ and CO$_2$. Roughton and Forster (1957) measured the
overall reaction rate of O\textsubscript{2} with hemoglobin and indicated its importance to the rate of gas exchange in the lungs. Earlier mathematical simulations of pulmonary gas exchange had generally not consider chemical reactions. For example, Defares and Visser (1962) and Milhorn and Pulley (1968) calculated changes in \( P_{O2} \) and \( P_{CO2} \) along the pulmonary capillaries on the basis of mixed venous values, diffusing capacities, and simplifying assumptions about the dissociation curve shapes. Neither of these studies attempted to consider chemical reaction rates. Wagner and West (1972) presented calculations which included CO\textsubscript{2} reaction rates as well as O\textsubscript{2} reaction rates and O\textsubscript{2}/CO\textsubscript{2} interactions. They allowed for the finite rate of chemical reactions of CO\textsubscript{2} by choosing representative values for the overall rate of CO\textsubscript{2} reaction with blood to predict changes in \( P_{O2} \) and \( P_{CO2} \) as function of time during the capillary transit. They also considered the effects of diffusion limitation and ventilation-to-perfusion inequality on pulmonary gas exchange. This was essentially a compartmental model in which a mean value of \( P_{O2} \) was used in the formulation.

Hill et al. (1973a) presented a computer simulation study of a model for the exchange of CO\textsubscript{2} and O\textsubscript{2} between fetal and maternal blood in the placenta, which they subsequently modified to describe pulmonary CO\textsubscript{2} and O\textsubscript{2} exchange (Hill et al., 1973b). It was a one-dimensional model of gas exchange that takes place in the lungs, and the role played by convection and diffusion in the transport of the gases was not taken into account. Rather than using an overall rate constant for CO\textsubscript{2}, as did Wagner and West, they included the commonly recognized reactions and processes, including the chloride shift and O\textsubscript{2}/CO\textsubscript{2} interactions. The reaction of CO\textsubscript{2} with hemoglobin was included in their studies, and the description of the anion exchange was based on an empirical relationship for the Gibbs-Donnan ratio. Incorporating exchange between cells and plasma, they predicted a slow readjustment of plasma \( pH \) and related effects after capillary transit. Calculations performed that assumed the rate of hydration of CO\textsubscript{2} in the
plasma to be equal to that in RBC did not change appreciably the total transfer rate of CO₂, although the reaction path was different. Sirs (1970) and Hill et al.'s findings were consistent with the experimental work of Forster and Crandall (1975).

In vitro confirmation of Roughton's postulate was provided by Forster and Crandall (1975) when they showed that the rate of pH equilibration in RBC suspensions was two orders of magnitude slower than the rate of uptake of CO₂ (half time of 2 and 0.045 sec, respectively). It was further shown that the slow readjustment of plasma pH can be eliminated by adding carbonic anhydrase to the plasma. They also considered a simplified model for describing the time course of exchange between RBCs and the extracellular fluid during O₂ uptake. This model included some of the chemical processes important in CO₂ uptake by blood but not the reaction of CO₂ with hemoglobin. In addition, the description of the exchange of anions between the plasma and RBCs in their model was based on the theory of electrodiffusion of ions (Goldman's solution to the Nernst-Planck equation which is given in Biophysical Chemistry of Membrane Functions by Kotyk et al., 1988). A slightly more elaborate model for pulmonary exchange was later on given by Bidani et al. (1978). Agreement of both catalyzed and uncatalyzed experiments with a simplified compartment model led them to conclude that pH does not reach its eventual equilibrium value in the lung. Result of experiments by Klocke (1973) also suggested that even dissolved CO₂ may not attain equilibrium during capillary transit.

Several investigators then designed experimental protocols to verify the presence of such slow pH changes in the arterial circulation in anesthetized animals. If plasma pH does not achieve chemical equilibration with P CO₂ and the bicarbonate system during transit through the lung, one would expect effluent blood pH to change with time. Using a stopped-flow pH apparatus, Hill et al. (1977) and Bidani et al. (1978) measured the pH of blood as a function of time after it had passed through the lungs and observed
a smaller than predicted \( pH \) change in blood leaving the lungs. Whereas in the laboratories of Crandall and O’Brasky (1978), Effros et al. (1978) and Klocke (1980), the question was approached from a different angle. Isolated lungs were perfused with fluid containing no hemoglobin or carbonic anhydrase and the effects on capillary exchanges observed. They observed no effluent perfusate \( pH \) disequilibrium which indicated that the perfusate \( CO_2 \) reactions was completed during the pulmonary capillary transits. Moreover, addition of carbonic anhydrase inhibitor (such as acetazolamide) to the perfusate yielded an increase in effluent \( pH \) with time. Consequently, they reached the conclusion that carbonic anhydrase was available to the perfusate in the lung capillaries and it was bound to the luminal surface of the endothelial cells. Carbonic anhydrase had been reported in cultured pulmonary endothelium (Ryan and Ryan, 1984) which provided the morphological support for such an enzyme location and distribution.

Salathe et al. (1981) presented a time-dependent study of acid/base balance in the microcirculation which involved the study of \( CO_2 \) transport and its relationship to \( O_2 \) transport, as well as a study of the buffer systems that are intimately connected to the transport of these gases. Their study did not take into account the transport of species due to diffusion, convection; and the exchange of \( HCO_3^- \) and \( Cl^- \) was regarded as passive electrodiffusion.

Singh et al. (1980) considered a model of a capillary slit exchanging \( O_2 \) and \( CO_2 \) through the plane walls. They formulated five balance equations for \( O_2 \), \( CO_2 \), Hb, HbO\(_2\) and HbCO\(_2\), and presented an order-of-magnitude analysis of the problem. These equations described convection of substances along the capillary, and facilitated transport of \( O_2 \) and \( CO_2 \) were included in the model. These researchers used the governing equations subsequently in a series of publications that presented numerical solution to the problem, took into account axial diffusion in the capillary and pulmonary membrane resistance, and considered unsteady processes (as summarized in Sharan et al., 1987).
However, their governing O₂-CO₂-hemoglobin reaction appeared to be based on the assumption that O₂ and CO₂ compete for the same sites on the hemoglobin molecules which is significantly different from the known mechanism of gas transfer by blood.

Based on Krogh tissue cylinder geometry, Reneau et al.'s (1967, 1969) models for O₂ transport in a systematic capillary included a constant metabolic rate, radial O₂ gradients in the capillary, and the nonlinear aspects of O₂ storage within the RBCs. Under steady-state conditions, the effect of the blood velocity profile was studied while ignoring the existence of axial diffusion in both the capillary and tissue. In addition, the effects of variations in arterial $P_{CO2}$ were also studied. The unsteady or transient situation of O₂ transport were created by allowing arterial $P_{O2}$ to increase suddenly by keeping other factors constant. It was seen that the reaction in the capillary predominates over convection and molecular diffusion during initial times.

McCracken et al. (1972) studied the interacting effects of O₂, CO₂ and glucose in the capillaries and tissue of the human brain. They did not consider the facilitated diffusion of the species and the kinetics of hemoglobin with O₂ and CO₂ in their model. They solved the system of interactive equations for describing the simultaneous transport of O₂, CO₂ and glucose and concluded that the interaction of components such as O₂ and CO₂ in the capillary blood is very important in the transport of O₂ to tissue. Artigue (1980) used McCracken et al.'s work as a basis for further investigation. He considered both the steady-state and dynamic models of transport of O₂, CO₂, glucose and lactic acid. The facilitated diffusion and the interactions of O₂ and CO₂ (the Bohr and Haldane effects) were taken into account with empirical equations which were developed based on experimental data. However, the convective diffusive equations were simplified by assuming local chemical equilibrium. The effects of blood pH on the dissociation curves for O₂ and CO₂ were not included. He attempted to take into account the two-phase nature of blood by considering the RBCs to be a separate phase which is uniformly
distributed in the blood according to the prescribed hematocrit. Diffusional resistance between the cells and the plasma was taken into account by a means of a mass transfer coefficient.

Baxley and Hellums (1983) developed a model for intracapillary transport in which they considered RBCs to be cylindrical slugs and included radial diffusion and reaction within the RBC and also included diffusion in the plasma gap between the RBCs and the capillary wall. They also took into account the effective flux from the RBCs due to the presence of alternate plasma gaps between them. Federspiel and Popel (1986) developed a somewhat different model for small capillaries in which they treated RBCs as spheres and took RBC spacing, RBC velocity and RBC clearance as parameters. They solved the problem of intracapillary O₂ transport numerically and presented the result in terms of a mass transfer coefficient. Both groups found an increased resistance due to the discrete nature of blood over hemoglobin solutions. There are other theoretical studies on intracapillary gas transport; however, none of these models have yet been validated by experiments.

These studies offered insight into the integrated changes which occur during physiological transfer. However, for a number of reasons, the results and conclusions may not apply directly when considering gas transport in microvessels and artificial membrane oxygenators. Although the steps involved for gas transport are the same, but the mass transfer and flow characteristics are significantly different. On one hand, the diffusional process is very efficient at nearly capillary dimensions; on the other hand, diffusion in blood becomes an inefficient means of transport and is known to limit gas transfer in large microvessels and macroscopic flow channels whose diameters are orders of magnitude greater than that of lung or tissue capillaries. Additionally, cellular level effects are somewhat masked in the large microvessels due to the fact that most of the transport resistance lies in the plasma phase. Therefore, a rigorous and detailed
description of intraerythrocytic activities is probably not necessary for the formulation of large microvessel models.

3.2 Mutual Transfer of Oxygen and Carbon Dioxide to and from Blood in Artificial Membrane Tubes

Mathematical models for describing the mass transfer of $O_2$ and $CO_2$ by blood flowing in blood vessels or artificial membrane tubes can be obtained from the species continuity equation which includes convection, diffusion and chemical reaction of various species in the blood. A general approach to the transfer of $O_2$ and $CO_2$ would require a three phase model of the blood with interphase exchange of various species. Therefore, equations for various species can be written for the RBC, RBC membrane and extracellular plasma phases. A material balance for species $i$ in phase $k$ can be written as:

$$\frac{\partial [C_i]_k}{\partial t} + \text{div} \left( \vec{V} [C_i]_k \right) = \nabla \cdot D_{i,k} \nabla [C_i]_k + R_{i,k} \tag{3.1}$$

where

$\vec{V}$ is the velocity vector.

$[C_i]_k$ is the concentration of species $i$ in phase $k$.

$D_{i,k}$ is the diffusion coefficient of species $i$ in phase $k$.

$R_{i,k}$ is the rate of reaction which generates $i$ in phase $k$.

The material balance of species $i$ inside the RBC phase would be coupled through continuity of flux and concentration of $i$ to the continuity equation of species $i$ for the RBC membrane phase at the inside surface of the membrane. Similarly, continuity of
equation of specie i for the plasma phase would be coupled to the equation for the RBC membrane at the outside surface of the membrane.

The simplified approach which had been used by most of the investigators who modeled O\textsubscript{2} and CO\textsubscript{2} transfer was to consider overall mass balances for O\textsubscript{2} and CO\textsubscript{2} that are applied to a combined RBC-RBC membrane-plasma continuum. In addition, the majority of analysis also considered steady state transfer and local chemical equilibrium. Thus Equation (3.2), written for one phase, is applicable, but the diffusion coefficients must be interpreted as effective values for the continuum to account for the heterogeneity of the blood.

\[
\sum_i \text{div} \left( \nabla [C_i] \right) = \sum_i \nabla \cdot D_i \nabla [C_i]
\]  

Equation (3.2) represents summation over the sets of equations obtained for various forms of O\textsubscript{2} and CO\textsubscript{2} in the blood individually; and it sums both total reaction for O\textsubscript{2} and CO\textsubscript{2} separately to be zero. Therefore, Equation (3.2) is applicable to both species, O\textsubscript{2} and CO\textsubscript{2}. Furthermore, assuming a tubular channel with axial symmetry and invoking assumptions (1) - (3), listed below, leads to Equations (3.3) and (3.4).

(1) There is negligible axial and angular diffusion.
(2) Transport by convection occurs only in the axial direction.
(3) Species i has constant diffusivity.

\[
V(r) \frac{\partial [O_2]_{\text{total}}}{\partial [O_2]} \frac{\partial [O_2]}{\partial z} = \frac{D_{O_2}}{r} \frac{\partial}{\partial r} \left[ \left( 1 + \frac{D_{HbO_2}}{D_{O_2}} \frac{\partial [HbO_2]}{\partial [O_2]} \right) \left( r \frac{\partial [O_2]}{\partial r} \right) \right]
\]  

\[ (3.3) \]

\[
V(r) \frac{\partial [CO_2]_{\text{total}}}{\partial [CO_2]} \frac{\partial [CO_2]}{\partial z} = \frac{D_{CO_2}}{r} \frac{\partial}{\partial r} \left[ \left( 1 + \frac{D_{HCO_3}}{D_{CO_2}} \frac{\partial [HCO_3]}{\partial [CO_2]} + \frac{D_{HbCO_2}}{D_{CO_2}} \frac{\partial [HbCO_2]}{\partial [CO_2]} \right) \left( r \frac{\partial [CO_2]}{\partial r} \right) \right]
\]  

\[ (3.4) \]
where

\( r \) and \( z \) are the radial and axial coordinates, respectively, of the tubular channel.

\( V(r) \) is the velocity profile in the \( z \) direction.

\( D_{O_2} \) and \( D_{CO_2} \) are the effective diffusivities of dissolved \( O_2 \) and \( CO_2 \) in the suspension, respectively.

\( D_{HbO_2} \) and \( D_{HbCO_2} \) are the effective diffusivities of oxyhemoglobin and carbamino hemoglobin in the suspension, respectively.

\( D_{HCO_3} \) is the effective diffusivity of bicarbonate ion in the suspension.

\([O_2]\) and \([CO_2]\) are the physically dissolved concentration of \( O_2 \) and \( CO_2 \) in the suspension, respectively.

\([HbO_2]\) and \([HbCO_2]\) are the concentrations of oxyhemoglobin and carbamino hemoglobin in the suspension, respectively.

\([HCO_3^-]\) is the concentration of bicarbonate ion in the suspension.

\([O_2]_{total}\) is the total \( O_2 \) content which includes both oxyhemoglobin and dissolved \( O_2 \).

\([CO_2]_{total}\) is the total \( CO_2 \) content which includes bicarbonate ion, carbamino hemoglobin, dissolved \( CO_2 \) and carbonic acid.

Here, the differential balances have been written in terms of the dissolved \( O_2 \) and \( CO_2 \), \([O_2]\) and \([CO_2]\). The convective terms in Equations (3.3) and (3.4) account for axial transport of all \( O_2 \) and \( CO_2 \) species (denoted by \([O_2]_{total}\) and \([CO_2]_{total}\), respectively). The diffusive terms account for only the major diffusing species - specifically, for \( O_2 \) there are dissolved \( O_2 \) and oxyhemoglobin; and for \( CO_2 \) there are dissolved \( CO_2 \), bicarbonate ion and carbamino hemoglobin. Again, the diffusion coefficients are effective values in the above development. The following review is presented in chronological order.
Buckles (1966) and Buckles et al. (1968)

Buckles and coworkers studied the steady-state transfer of $O_2$ to flowing blood, using a straight silicone rubber membrane tube system. Their analysis was based on a microscopic local equilibrium model, but involved the convective terms and even incorporated a correction for non-Newtonian flow effects. The continuity equation for $O_2$ is given in Equation (3.5), except for the fact that Buckles et al. used the Casson model for the velocity profile. Buckles also did a full analysis of the gas transport through the wall and considered the wall flux as one of the boundary conditions. Experimental measurements of $O_2$ uptake by deoxygenated (or reduced) blood at 38 °C and constant $P_{CO_2}$ agreed moderately well with the transfer rates predicted by the numerical solution of Equation (3.5), using an effective value of $D_{O_2}=1.4\times10^{-5}$ cm$^2$/sec, although the data did not follow the shape of the theoretical curve closely.

Weissman and Mockros (1967 and 1969)

The $O_2$ transport model developed by Weissman and Mockros is very similar in nature to the model developed by Buckles (1966). Weissman and Mockros' $O_2$ transport differential equation, transformed to the symbols used elsewhere in this paper, is given below. Equation (3.5) was obtained from Equation (3.3) by further introducing the approximation that the diffusion of oxyhemoglobin is sufficiently small relative to the diffusion of $O_2$ to be neglected.

\[
V(r) \left( 1 + \frac{d[HbO_2]}{d[O_2]} \right) \frac{\partial [O_2]}{\partial z} = \frac{D_{O_2}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]}{\partial r} \right)
\]

(3.5)

where

$V(r)$ is a parabolic velocity profile in tubes. Because the non-Newtonian nature of the blood, the velocity profile should be more appropriately described by an integrated form of Casson equation. However, the Casson model approximates a parabolic profile
in the flow range of conditions investigated by these investigators; therefore, a simple parabolic Newtonian type model is reasonable here.

\[ \frac{d[HbO_2]}{d[O_2]} = \frac{C_{heme,total}}{\alpha_{O_2}} \frac{dS}{dP_{O_2}} \]  

(3.6)

where \( S \) is the fraction of the total hemoglobin \( O_2 \) binding sites that are occupied by \( O_2 \); \( C_{heme,total} \) is the total heme concentration in the medium; \( \alpha_{O_2} \) is the Bunsen solubility coefficient \( O_2 \) in blood; and \( P_{O_2} \) is the partial pressure of \( O_2 \). \( dS/dP_{O_2} \) is the slope of the ODC. Weissman and Mockros obtained the description of the relationship between \( S \) and \( P_{O_2} \) by fitting an exponential function to the ODC.

They also ignored the effect of the wall on the overall gas transport rate; i.e., they neglected the mass transfer resistance of the wall. The boundary conditions which were imposed for this problem are

\[ [O_2] = [O_2]_o \]  

at \( z = 0 \) for \( 0 \leq r \leq r_c \)  

(3.7a)

\[ \frac{\partial [O_2]}{\partial r} = 0 \]  

at \( r = 0 \) for \( z > 0 \)  

(3.7b)

\[ [O_2] = [O_2]_\infty \]  

at \( r = r_c \) for \( z > 0 \)  

(3.7c)

The term, \( d[HbO_2]/d[O_2] \), makes this equation nonlinear. Any attempt at linearization, such as replacing the slope of ODC by a constant, for instance, eliminates a major aspect of the problem; thus the above system of equations, Equations (3.5) - (3.7), was solved numerically. Weissman and Mockros did not predict a priori an \( O_2 \) diffusivity for blood.
Instead, for each experiment they calculated the appropriate diffusivity which would bring the solution of their equations close to agreement with the experimental findings. In addition, the experimental device which they used was an oxygenator alone which did not simulate the lung’s function; a constant CO₂ partial pressure was maintained to prevent simultaneous diffusion of CO₂.

As an extension, Weissman and Mockros used a simplified form of Equation (3.4) to theoretically predict CO₂ removal in a permeable membrane tube. They neglected the diffusion of bicarbonate ion and carbamino hemoglobin; and assumed that in the physiological pressure range [CO₂]_{total} and [CO₂] for arterial blood are related through the following linear relationship

\[
[CO₂]_{total} \left( \text{mole\ per\ liter} \right) = 1.14 \times 10^{-2} + 10.0 \ [CO₂]
\] 

(3.8)

The slope of total CO₂ versus dissolved species relationship was given as \( d[CO₂]_{total} / d[CO₂] = 10 \). In addition, only diffusion of dissolved CO₂ was considered; thus Equation (3.4) was simplified to the following

\[
10 \ V(r) \ \frac{\partial[CO₂]}{\partial z} = \frac{D_{CO₂}}{r} \ \frac{\partial}{\partial r} \left( r \ \frac{\partial[CO₂]}{\partial r} \right)
\] 

(3.9)

Equation (3.9) is a linear partial differential equation and the boundary conditions selected were similar to those that are imposed on the O₂ transfer problem, Equations (3.7a) - (3.7c). The solution to this boundary problem, a nonreactive problem for the tubular geometry, was given by Graetz’s solution. However, no experimental corroboration were offered to verify the CO₂ transport model. Therefore, Weissman and Mockros’ model did not allow the influence of CO₂ and H⁺ content of the blood on the O₂ transport (the Bohr effect) and vice versa the impact of oxygen saturation on the CO₂
transport (the Haldane effect). They neglected the interdependence of \(O_2\) and \(CO_2\) transport and decoupled the problem into two independent transfer processes.

Bradley (1969); Bradley and Pike (1971)

Bradley and Pike also considered \(CO_2\) transport in the presence of simultaneous \(O_2\) transport in a tubular geometry. Two models were developed, and the first one considered the steady state oxygenation as independent of \(CO_2\) removal. The second model considered \(CO_2\) transfer on the simultaneous \(O_2\) transfer. The resulting convective-diffusive equations for \(O_2\) and \(CO_2\) were similar to that obtained by Weissman and Mockros; except for the fact that the influence of a changing \(O_2\) saturation on the \(CO_2\) transfer was included. Bradley and Pike modeled the ODC by an exponential fit. In addition, they also assumed a linear relationship between total \(CO_2\) content and \(P_{CO2}\) and included a linear correction for oxygen saturation to account for the Haldane effect.

\[
[CO_2]_{total} \left( \text{moles liter}^{-1} \right) = 1.67 \times 10^{-2} - 3.34 \times 10^{-3} S + 2.04 \times 10^{-4} P_{CO2} 
\]  

(3.10)

This correlation was reported for \(T\) at 38 °C and hematocrit of 45%, and \(P_{CO2}\) is the partial pressure of \(CO_2\) in mmHg.

The equation for \(CO_2\) transport which is given by Equation (3.11) was solved numerically and simultaneously with an \(O_2\) transport equation.

\[
V(r) \left[ \frac{2.04 \times 10^{-4}}{\alpha_{CO2}} \frac{\partial [CO_2]}{\partial z} - 3.34 \times 10^{-3} \frac{\partial S}{\partial z} \right] = \frac{D_{CO2}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [CO_2]}{\partial r} \right) 
\]  

(3.11)

where \(\alpha_{CO2}\) is the effective solubility coefficient for \(CO_2\) in the suspension. Boundary conditions, Equations (3.7a) and (3.7b) were used. Furthermore, they relaxed the
assumption of negligible wall resistance made by Weissman and Mockros which would not be valid for membrane tubes that are not as permeable and/or have a fairly high wall thickness to inner diameter ratio. They introduced the flux matching boundary condition

\[ D_{CO2} \frac{\partial [CO2]}{\partial r} = D_{CO2,m} \frac{\partial [CO2]_m}{\partial r} \] at \( r = r_c \) for \( z > 0 \) (3.12)

where

- \( D_{CO2,m} \) is the diffusion coefficient of CO2 in the membrane.
- \([CO2]_m\) is the concentration of CO2 in the membrane.

Although data was taken using only partial pressure measurements, there was large scatter in the results. In addition, Bradley and Pike's model predicted the amount of CO2 transferred to be less than that found experimentally. The fact that the model underpredicted the measured transfer rates might be due to the neglect of simultaneous diffusion of CO2 in the form of bicarbonate ion.

**Villarroel (1970) and Villarroel et al. (1971)**

In continued studies of the gas exchange processes with flowing blood in membrane system, Villarroel et al. explored the effect of steady versus pulsatile flow conditions, using a macroscopic model similar to that used by Weissman and Mockros but incorporating non-Newtonian flow effects and accounting for the tube wall resistance. They found that use of the Casson equation was not necessary for the steady-state flow range studied. Therefore, a parabolic Newtonian velocity profile was incorporated for the steady-state flow conditions, and a flux matching boundary condition was employed. The ODC was curve fitted with a function which consists of two dependent variables, \( P_{CO2} \) and \( pH \). The total CO2 content approximation used was that of Weissman and Mockros (Equation (3.8)). Therefore, only the fixed acid Bohr
effect was accounted for here and the CO₂ Bohr effect and the Haldane effect were neglected. As with previous investigators, bicarbonate ion diffusion was neglected. In addition, they provided for shear augmented diffusion but found this unnecessary for the flow range studied.

A numerical solution to this model was developed which allowed both O₂ and unfacilitated CO₂ profiles to be computed simultaneously. This, in turn, permitted the evaluation of the pH profile in the tube using the standard Henderson-Hasselbalch equation; however, the gross assumption that HCO₃⁻ was constant in the vessel was utilized for the above calculation. The ODC could then be corrected to these local conditions during the numerical integration. The application of this more detailed, coupled macroscopic model to experimental data obtained by previous workers, Buckles (1966) and Weissman and Mockros (1967), and their own data was found to result in improved fits. However, it should be mentioned that all those experiments considered only O₂ transfer in isolation because a constant CO₂ partial pressure was maintained to prevent simultaneous diffusion of CO₂.

**Fair and Weissman (1971)**

Fair and Weissman modified the earlier Weissman and Mockros' straight tube theory to include the interrelationship of O₂ storage in the blood and the amount of CO₂ in the blood (the Bohr effect) and of CO₂ storage to the amount of O₂ in the blood (the Haldane effect). Empirically fit ODC including the Bohr effect and CO₂ buffer equation including the Haldane effect were used. However, a constant wall concentration was employed again. In order to avoid numerical difficulties in the solution, the wall concentration was chosen as a nominal small value (corresponding to P_{CO₂}=1 mmHg). By comparison with prior work of Weissman and Mockros (i.e., no coupling), it was shown that the inclusion of the Bohr and Haldane effects (i.e., coupling) did result in
changes in uptake rates; however, these are only on the order of ± 20% and thus considered to be of secondary importance.

Dorson et al. (1971)

Dorson et al. treated blood as an overall continuum which has only dissolved and reacted CO₂ species in local equilibrium, and for tube flow at steady state the convective removal is balanced against the effective radial diffusion as represented by Equation (3.4). They neglected the carbamino hemoglobin’s contribution to diffusion on the basis of its large molecular weight. Additionally, they assumed that the relationships of total CO₂ content and bicarbonate ion with dissolved CO₂ can be approximated in a linear form:

\[
[CO₂]_{total} = E_i + F_s[CO₂] \quad (3.13a)
\]

\[
[HCO₃] = G_i + H_s[CO₂] \quad (3.13b)
\]

where

\(E_i\) and \(F_s\) are the intercept and slope, respectively, of linearized dissolved CO₂ content versus total CO₂ content curve.

\(G_i\) and \(H_s\) are the intercept and slope, respectively, of linearized dissolved CO₂ content versus HCO₃⁻ content curve.

Upon introducing these additional assumptions, Equation (3.4) becomes

\[
V(r) \frac{\partial[CO₂]}{\partial z} = \frac{D_{CO₂}}{F_s} \left( 1 + \frac{D_{HCO₃}}{D_{CO₂}} H_s \right) \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[CO₂]}{\partial r} \right) \quad (3.14)
\]

Along with the standard conditions which include Equations (3.7a) and (3.7b), the wall flux matching condition was used to determine the variable wall concentration:
\[ D_{CO2} \left( 1 + \frac{D_{HCO3}}{D_{CO2}} H_s \right) \frac{\partial [CO_2]}{\partial r} = D_{CO2,m} \frac{\partial [CO_2]_m}{\partial r} \] (3.15)

Therefore, the entire complexity of multiple reacting and diffusing species is lumped into the ratio, \( D_{CO2} \left( 1 + \frac{D_{HCO3}}{D_{CO2}} H_s \right) / F_s \), and the flux matching boundary condition, Equation (3.15).

Comparison of the model calculations with their experimental data were not completely satisfactory. A basic problem was that this approach relied on appropriate slope determinations for estimating the values of \( F_s \) and \( H_s \). When blood acid/base was altered significantly from standard condition, the generalized charts that they used were of little value. In addition, for small wall resistances, steep internal CO₂ profiles further complicated selection of \( F_s \) and \( H_s \). Furthermore, for large CO₂ transfer rates, a linear approximation of the total CO₂ content and HCO₃⁻ content versus \([CO_2]\) became a poor assumption. However, they did report that the comparison was improved when measured slopes were used.

Chen (1972)

Chen theoretically studied CO₂ transfer in a single permeable tube. Total content curves were generated by using a published nomogram in conjunction with the plasma \( pH \) and plasma bicarbonate relationship. Numerical results were computed with and without bicarbonate ion diffusion. The relationship between the dissolved species and the convected and diffused reacted species were expressed mathematically as implicit derivatives which multiplied the convective and diffusive terms. He concluded that if bicarbonate ion diffusion occurred, it would significantly enhance CO₂ transfer. However, no data were reported to support this contention.

Sosa and Merchuck (1972)

This group of researchers studied the gas transfer in a film of blood flowing through a rectangular membrane artificial lung. Their mathematical analysis treated blood as a homogeneous, non-Newtonian fluid, in which the system \( P_{O2}, P_{CO2} \) and \( pH \)
were in equilibrium due to the reaction rate in the RBCs. They used more complicated empirical expressions to account for the interdependence of O₂ and CO₂ transport. For instance, the extent of hemoglobin saturation was fitted to be a function of the partial pressures of O₂ and CO₂, temperature and pH in the following form:

\[ S = \frac{(a_1 w + a_2 w^2 + a_3 w^3 + w^4)}{(a_4 + a_5 w + a_6 w^2 + a_7 w^3 + w^4)} \]  \hspace{1cm} (3.16)

where

\[ a_1, \ldots, a_7 \] are constants given by Kelman (1966).

\[ w \] is a corrected \( P_{O2} \) given by the following equation:

\[ w = P_{O2} \left[ 10^{0.024(37 - T) + 0.04(pH - 7.4) + 0.06(log_{40} - log_{P_CO2})} \right], \]

The pH of blood was given as the following

\[ pH = 8.059 + 0.012(1 - S) + 0.011(1 - S)C_{\text{heme, total}} \]

\[ - \left[ HCO_3^- \right] \left( \frac{0.02}{C_{\text{heme, total}}} \left[ 0.273 - 0.01(1 - S) \right] \right) \] \hspace{1cm} (3.17)

where \([HCO_3^-]\) is the concentration of bicarbonate ion in the blood and is derived from the Henderson-Hasselbalch equation: \([HCO_3^-] = \alpha_{CO_2} P_{CO_2} 10^{(pH - pK')}, \) and \( K' \) is the apparent first dissociation constant of carbonic acid and includes a factor which allows for the substitution of total dissolved CO₂ concentration for carbonic acid.

They used these analytical approximations for O₂ and CO₂ dissociation curves and solved the following two coupled partial differential equations:

\[ V_z \left[ 1 + C_{\text{heme, total}} \frac{\partial S}{\partial (\alpha_{O2} P_{O2})} \right] \frac{\partial (\alpha_{O2} P_{O2})}{\partial z} = D_{O2} \frac{\partial^2 (\alpha_{O2} P_{O2})}{\partial x^2} \]  \hspace{1cm} (3.18)
\[ V_z [1 + 10^{(\rho H - \rho K)}] \frac{\partial (\alpha_{CO2} P_{CO2})}{\partial z} = D_{CO2} \frac{\partial^2 (\alpha_{CO2} P_{CO2})}{\partial x^2} \]  
(3.19)

where \(x\) and \(z\) are the spatial coordinates of their experimental system. A flux boundary condition resulting from the mass balance of either \(O_2\) or \(CO_2\) at the blood membrane interface was used; the suffix \(i\) indicates either \(O_2\) or \(CO_2\).

\[ D_i \alpha_i \frac{\partial P_i}{\partial x} = \frac{D_{i,m} \alpha_{i,m} (P_{i,ext} - P_i)}{d} \]  
(3.20)

where

- \(P_i\) and \(P_{i,ext}\) are the gas pressures in the blood side and outside of the membrane, respectively.
- \(\alpha_i\) and \(\alpha_{i,m}\) are the solubility coefficients of the gas in the blood and in the membrane material, respectively.
- \(d\) is the thickness of the membrane film.

The authors reported that calculated values of oxygen saturation agree with the experimental data to within an error of 5-10%; however, it should be mentioned that there was some scatter in the results. In addition, no \(CO_2\) transport related quantities, such as \(P_{CO2}\) and \(pH\) were reported for the validation of the entire model. Furthermore, there is question to the applicable operating regime of the correlations they used.

Villarroel and Lanham (1973)

Villarroel and Lanham proposed a design method for tubular membrane oxygenators which included a modified version of their earlier models. Although bicarbonate diffusion was neglected, they employed a normal \(CO_2\) dissociation curve by using experimental data which accounted for the Haldane effect. The flux matching boundary condition was used, and data in the form of bulk exit \(P_{CO2}\) values were
reported. There was good agreement between the data and theory on a $P_{O_2}$ basis, but the agreement was due in part to the wall limited nature of their experimental devices. Total CO$_2$ transfer rates were reported.

**Benn (1974) and Benn *et al.* (1975)**

Benn and colleagues conducted a study of CO$_2$ transfer from weak acids and blood flowing in permeable tubes. They included the facilitation of CO$_2$ transport by bicarbonate ion, the kinetics of carbonic anhydrase (assumed to be uniformly distributed throughout the blood), and the effects of the predominant weak acids present in blood. Their experimental results suggested that dehydoration of carbonic acid could be treated as being at equilibrium. However, the sensitivity of the measurements to a departure from equilibrium of the carbonic anhydrase reaction was diminished by a significant membrane mass transfer resistance for CO$_2$.

CO$_2$ transfer theory in flowing blood was modeled by these researchers as an extension of the theory that they developed for their weak acid study. Blood was considered to be a homogeneous fluid composed of three CO$_2$ species; dissolved CO$_2$, bicarbonate ions, and carbamino-bound CO$_2$. Again, Equation (3.1) applies for each species, but was written only for dissolved CO$_2$ and bicarbonate ion.

$$\vec{V} \cdot \nabla [C_i] = D_i \nabla^2 [C_i] + R_i$$  \hspace{1cm} (3.21)

Carbamino CO$_2$ was incorporated into the dissolved CO$_2$ equation by assuming it to be in equilibrium with the dissolved CO$_2$ everywhere in the blood channel. A lumped buffering behavior of all weak acids was assumed, and a simplified reaction between bicarbonate and dissolved CO$_2$ similar to Reactions (2.I) and (2.II) was used. O$_2$ linked effects were included via simultaneous solution of an O$_2$ convection equation. Major conclusions from their work were
1. Diffusion of weak acids was negligible since hemoglobin, the major weak acid in blood, does not diffuse.

2. Data verified CO₂ transfer theory when the O₂ linked effects are minimal.

3. Under normal artificial lung operating conditions, their model predicted a 15% increase in CO₂ transport due to O₂ related effects. However, the difficulty of measuring simultaneous O₂ and CO₂ transfer to with an error less than 15% prevented experimental validation of O₂ enhanced CO₂ transfer.

Voorhees (1976)

Voorhees attempted to improve upon Dorson et al.'s (1971) model by treating the CO₂ equilibrium with blood differently. Basically, Equation (3.22) was used to relate the dissolved and reacted forms of CO₂:

\[
[CO₂] = \frac{[CO₂]_{reacted}}{K_{eq}} \times 10^{[\frac{[CO₂]_{reacted}}{\beta}]}
\]  

(3.22)

where

\[ [CO₂]_{reacted} \] is the concentration of reacted CO₂ in blood.

\[ K_{eq} \] is a pseudo equilibrium constant in approximate CO₂ dissociation curve which was determined from experimental measurements.

\[ \beta \] is the apparent buffering capacity of the blood which was determined semi-empirically.

Knowing \( K_{eq} \) and \( \beta \), all values of \( [CO₂] \) and \( [CO₂]_{reacted} \) can then be related through Equation (3.22). With the assumption concerning the relationship between the dissolved and reacted CO₂ and assumptions (1) - (3), listed previously for the derivation of Equation (3.4), Equation (3.2) can then be expressed as
\[ V(r) \frac{\partial}{\partial z} \left( [CO_2] + [CO_2]_{\text{reacted}} \right) = \frac{D_{CO_2}}{r} \frac{\partial}{\partial r} \left[ r \frac{\partial}{\partial r} \left( [CO_2] + \frac{D_{CO_2,\text{reacted}}}{D_{CO_2}} [CO_2]_{\text{reacted}} \right) \right] \] (3.23)

where \( D_{CO_2,\text{reacted}} \) is the effective, lumped diffusivity of reacted CO_2. In addition, the advancing front concept (Dorson and Voorhees, 1974) which decoupled the convective and diffusive contribution was applied to the O_2 transfer problem. Finally, his treatment of O_2, CO_2 equilibrium with blood was consistent with the assumption of blood being a homogeneous fluid. However, those correlations overlooked some of the subtle aspects of O_2-CO_2-blood interactions which included the Bohr and Haldane effects.

Agreement between data and theory for CO_2 transfer from blood was good for experiments to which carbonic anhydrase was added to the plasma portion of the sample. This further confirmed the validity of the homogeneous, local chemical equilibrium model developed by Voorhees. However, for most cases, CO_2 transfer data was over predicted by theory. This overprediction could be attributed to the fact that the model was underestimating the transport resistance by assuming the CO_2 hydration/dehydration to be in equilibrium in the plasma. For the cases of CO_2 transfer with either simultaneous oxygenation or deoxygenation and addition of extracellular carbonic anhydrase, the theory under calculated the amount of transport. This underprediction could be linked to the factor that the Haldane effect was not taken into account.
CHAPTER 4

OXYGEN TRANSPORT MODELS

There has been considerable activity in past years on the mathematical simulation of oxygen transport in the microcirculation. Various investigators have used different sets of assumptions to reduce the complex governing equations to a tractable set. Although there were experimental data available for the larger diameter microvessels (several hundred microns in diameter) from artificial membrane oxygenators, previously there has been insufficient experimental validation of the various assumptions for applying to the smaller diameter microvessels (less than 100 μm in diameter). Recently, Boland et al. (1987) have developed an in vitro microvessel microspectrophotometer system which allows accurate determination of oxygen fluxes to and from RBC suspensions in small cylindrical conduits (= 30 μm in diameter) under physiologically relevant conditions. In this system flow, transport and geometrical parameters can be controlled and measured accurately. Thus, validation of the various proposed models over a wider range of diameters has become possible.

Nair et al. (1989) have developed a small microvessel model which is entirely predictive. That is to say all parameters in the model are determined from the literature or by other means independent of capillary oxygen transport experiments. In addition, the model is discrete in the sense that the particulate (two-phase) nature of blood is considered. The model was shown to be in excellent agreement with the experimental oxygen transport results from 27 μm diameter artificial membrane tubes (Nair et al., 1989), as well as with deoxygenation results obtained by Schmukler and Chien (1985) in 100 μm vessels. The first part of this chapter can be regarded as an extension of Nair
et al.'s model in which the results of their study is used as a guide to formulate a much simpler discrete model.

Another objective of this chapter is both to further investigate the feasible diameter regime for application of the continuum (assuming that blood is a continuous and homogeneous hemoglobin solution) and discrete models and to assess the importance of the shear-induced augmentation on oxygen transport as a function of tube diameter, shear rate and oxygen storage potential of the blood. For this previously developed mathematical models of oxygen transport by flowing blood were adapted to model the in vitro experimental systems of Diller et al. (1980), Schmukler and Chien (1985) and Boland et al. (1987).

4.1 Mathematical Models

4.1.1 Discrete Models

In the case of uptake, oxygen must diffuse through the artificial membrane medium (in vitro situation) or the tissue region (in vivo situation), then convect and diffuse through the blood plasma. Subsequently, it diffuse through the red cell membrane; once it is inside the red blood cell, oxygen simultaneously diffuses and reacts in the hemoglobin solution ("facilitated diffusion"). In the case of release, the same processes occur in the reverse order. The approach is to estimate each of the resistances to oxygen transport and use these estimates with the appropriate differential equations of change in a model which yields the O_2 concentration distribution. First, for continuity the main features of Nair et al.'s model (1989) is briefly recapitulated. Then it will be shown that by introduction of certain well-founded assumptions, Nair et al.'s model which consists a system of four simultaneous, nonlinear partial differential equations can be reduced to one partial differential equation (PDE). The comparison
given in section 4.3.1 will show the two methods give essentially equivalent results and are in good agreement with experiments.

4.1.1.a Recap of Nair et al.'s Discrete Model

A brief outline of the Nair et al.'s model is given below. More detail is given by Nair (1988) and Nair et al. (1989). The red cell suspension is treated as two continuous, coexisting phases: a RBC phase and a plasma phase. Intracellular diffusion and reaction resistances are estimated by solution of the transport equations for a typical RBC under a prescribed O$_2$ flux at the RBC wall. The extracellular diffusion boundary layer resistance is obtained from the low Reynolds' number solution for mass transfer from a disc to fluid flowing past it. The model takes into account convection of both the plasma and RBCs, and radial diffusion in the plasma. The equations for O$_2$ transport by the RBCs are:

\[
V_{rbc}(r) \frac{\partial [O_2]_{rbc}}{\partial z} = - \left( \frac{S}{V_{rbc}} \right) \text{Flux}_{O2} + C_{heme,rbc} F([O_2]_{rbc}, S) \quad (4.1)
\]

\[
V_{rbc}(r) \frac{\partial (C_{heme,rbc}S)}{\partial z} = - C_{heme,rbc} F([O_2]_{rbc}, S) \quad (4.2)
\]

where

\[C_{heme,rbc} F([O_2]_{rbc}, S)\] is the reaction rate expressed as a rate of O$_2$ appearance per unit volume of RBC. It can be treated by any of the variable-rate-coefficient methods (Moll, 1969; Clark et al., 1985; Vandegriff and Olson, 1984b).

\[C_{heme,rbc}\] is the total heme concentration in RBC, a constant.

\([O_2]_{rbc}\) is the mean dissolved O$_2$ concentration in the RBC. \([O_2]_{rbc}\) is a value averaged within each individual RBC but varying with position of the RBC in the vessel.

\(S\) is the mean fraction O$_2$ saturation of the hemoglobin molecules.
\((s/v)_{rbc}\) is the surface to volume ratio of the RBC.

Flux\(_{O2}\) is the \(O_2\) flux crossing the RBC wall, a function of \(r\) and \(z\).

In the plasma only one species - dissolved oxygen is considered.

\[
(1 - h(r)) V_{pl}(r) \frac{\partial [O_2]_{pl}}{\partial z} = \frac{D_{O2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{pl}}{\partial r} \right) + h(r) \left[ \frac{\partial}{\partial r} \right] \text{Flux}_{O2} \quad 0 \leq r \leq r_r
\]  

(4.3)

\[
V_{pl}'(r) \frac{\partial [O_2]_{pl}'}{\partial z} = \frac{D_{O2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{pl}'}{\partial r} \right) \quad r_r \leq r \leq r_c
\]  

(4.4)

where

\(D_{O2,pl}\) is the diffusivity of \(O_2\) in the plasma.

\([O_2]_{pl}\) and \([O_2]_{pl}'\) are the dissolved \(O_2\) concentrations in the RBC-rich and RBC-free plasma region, respectively.

The velocity variables used here may be thought of as profile variables. Different velocity variables are sometimes used in flow of heterogeneous fluids. Specifically, \(h(r)V_{rbc}(r)\) gives the local volumetric flux of RBCs (volume of RBCs per unit time per unit area). Similarly \((1-h(r))V_{pl}(r)\) is the local volumetric flux of plasma. Velocity profiles for the RBCs and the plasma, \(V_{rbc}(r), V_{pl}(r)\) and \(V_{pl}'(r)\); radial hematocrit, \(h(r)\); and the method for estimation of the cell-free layer thickness are summarized in section 2.2.1.

At the RBC-rich and RBC-free region interface, continuity of oxygen tensions and fluxes is imposed; and the symmetry condition is used at the center of vessel. In the \textit{in vitro} experimental systems of Diller et al. (1980) and Schmukler and Chien (1985), the cylindrical vessel is embedded in the center of a film with circular cross section; in Boland et al.'s (1987), the microvessel is embedded in a thin film of rectangular section.
For simulating the experimental results, transport in the outer membrane film is incorporated as a boundary condition at the vessel wall. Therefore, the steady-state asymptotic solution of the Laplace equation in the film yields the vessel wall O\textsubscript{2} flux (Balcerzak and Raynor, 1961):

\[-D_{O2,pl} \frac{\partial \left[ O_2 \right]_{pl}}{\partial r} \bigg|_{r=r_c} = \frac{K_{O2}}{\alpha_{O2,pl} \ln(GF)} \left( \left[ O_2 \right]_{pl} \big|_{r=r_c} - \alpha_{O2,pl} P_{O2,ext} \right)\]

(4.5)

where

\( K_{O2} \) is the oxygen permeability of the silicone rubber film.

\( \alpha_{O2,rbc} \) and \( \alpha_{O2,pl} \) are the solubility coefficients for O\textsubscript{2} in the RBC and the plasma, respectively.

\( GF \) is the geometric factor. \( GF=r_o/r_c \), where \( r_o \) is the outer radius of the membrane oxygenator, in both Diller et al.'s and Schmukler and Chien's systems; and \( GF=4d/\pi r_c \), where \( d \) is the half-sheet thickness of the silicone rubber film in Boland et al.'s system.

Finally, to complete the description, inlet conditions are imposed at \( z=0 \) where \( [O_2]_{rbc}, S, [O_2]_{pl}, \) and \( [O_2]'_{pl} \) are assumed to be in equilibrium and uniform with respect to radius. Therefore, Nair et al.'s model consists of a system of four simultaneous nonlinear PDEs which determine the O\textsubscript{2} transport variables \( [O_2]_{rbc}, [O_2]_{pl}, [O_2]'_{pl} \) and \( S \) as functions of \( r \) and \( z \) for given values of the various parameters.

4.1.1.b **Simplified Discrete Model (Simplified DM)**

Nair et al.'s discrete model revealed that within the 27 \( \mu \)m diameter microvessel, only about 2% of the resistance lies inside the RBCs, and only another 2% is associated with the plasma boundary layer close to the RBC wall. Thus, some 96% of the intracapillary resistance is distributed in the plasma - not inside or directly adjacent to the
cells. Furthermore, for larger microvessels the resistance associated with the red cells is even less important. Since the resistances attributed to the RBC interior and the diffusion boundary layers close to the RBC wall are small, the assumptions of chemical equilibrium within the red cell and negligible intracellular and cell boundary layer resistances can be introduced to simplify Nair et al.'s model.

By combining Equations (4.1) and (4.2) and invoking the chemical equilibrium assumption, one obtains

\[
V_{rbc}(r) \left( 1 + \frac{C_{\text{heme},rbc}}{\alpha_{O_2,rbc}} \frac{dS}{dP_{O_2}} \frac{\partial [O_2]_{rbc}}{\partial z} \right) = - F \mu x_{O_2}
\]

(4.6)

Since chemical equilibrium within the RBC is assumed, the fractional saturation of hemoglobin, \( S \), can be expressed as a function of the \( O_2 \) concentration, \([O_2]_{rbc}\). The term \( dS/dP_{O_2} \) is the slope of the oxyhemoglobin dissociation curve and is a nonlinear function of \( P_{O_2} \). It is also assumed that there is no transport resistance in the RBC membrane; thus the oxygen tension is continuous at the RBC wall (i.e., \([O_2]_{rbc, rw}/\alpha_{O_2, rbc} = [O_2]_{pl, rw}/\alpha_{O_2, pl}\); where \([O_2]_{rbc, rw}\) and \([O_2]_{pl, rw}\) are the \( O_2 \) concentrations at the RBC wall in the RBC and in the plasma, respectively). In addition, if the intra- and extra-cellular boundary layer resistances are neglected (i.e., \([O_2]_{rbc} = [O_2]_{rbc, rw}\) and \([O_2]_{pl} = [O_2]_{pl, rw}\)), then it follows that

\[
[O_2]_{pl} = \frac{\alpha_{O_2, pl}}{\alpha_{O_2, rbc}} [O_2]_{rbc}
\]

(4.7)

Multiplication of Equation (4.6) by \( h(r) \), addition of the resultant expression to Equation (4.3) and substitution of \( (\alpha_{O_2, pl}/\alpha_{O_2, rbc})[O_2]_{rbc}\) for \([O_2]_{pl}\) yields the following mass balance for dissolved \( O_2 \) in the RBC:
\[
\left(1 - h(r)\right) V_{pl}(r) + \left(\frac{\alpha_{O_2, rbc}}{\alpha_{O_2, pl}}\right) h(r) V_{rbc}(r) \left[ 1 + \frac{C_{heme, rbc}}{\alpha_{O_2, rbc}} \frac{dS}{dP_{O_2}} \right] \frac{\partial [O_2]_{rbc}}{\partial z} = \left(\frac{D_{O_2, pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{rbc}}{\partial r} \right) \right) 0 \leq r \leq r_r \quad (4.8)
\]

The plasma \(O_2\) concentration in the cell-rich region is obtained through Equation (4.7).

To further simplify Nair et al.’s model, the mass balance for dissolved \(O_2\) in the cell-free region can be solved analytically and incorporated as a boundary condition to Equation (4.8). This solution uses the finding that convective transport of \(O_2\) in the cell-free plasma region is small relative to transport by molecular diffusion. Hence, the term \(V_{p}'(r) \frac{\partial [O_2]_{pl}}{\partial z}\) can be dropped from Equation (4.4). The simplified equation can be integrated and expressed in terms of the wall flux or in terms of the flux from the cell-rich region (Equation (4.9)):

\[
- D_{O_2, pl} \frac{\partial [O_2]_{pl}}{\partial r} \bigg|_{r=r_r} = - D_{O_2, pl} \left( [O_2]_{pl}|_{r=r_c} - [O_2]_{pl}|_{r=r_r} \right) \frac{r_r}{r_r ln\left(\frac{r_c}{r_r}\right)}
\quad (4.9)
\]

The vessel wall oxygen flux is the product of the left hand side of Equation (4.9) and the ratio, \(r_r/r_c\). Equation (4.9) can easily be incorporated into the boundary conditions of the \(O_2\) transport problem. For example, in the simplest case of specified vessel wall \(O_2\) concentration, Equation (4.9) as it stands is the boundary condition on Equation (4.8). In applying these conditions, expression in terms of \([O_2]_{rbc}\) instead of \([O_2]'_{pl}\) is derived from use of Equation (4.7) and from conditions of continuity \([O_2]_{pl}=[O_2]'_{pl}\) and \(\partial [O_2]_{pl}/\partial r=\partial [O_2]'_{pl}/\partial r \) at \(r=r_r\).

In the particular case of the boundary condition for the artificial membrane tube system, Equation (4.5), the usual summation of resistance approach gives:
\[
- D_{O_2,pl} \frac{\partial [O_2]_{rbc}}{\partial r} \bigg|_{r=rr} = - \frac{D_{O_2,pl} (\alpha_{O_2, rbc} P_{O_2,ext} - [O_2]_{rbc} \bigg|_{r=rr})}{r_r \left[ \frac{D_{O_2,pl}}{KO_2} \frac{\alpha_{O_2,pl} ln(GF)}{KO_2} + ln \left( \frac{r_c}{r_r} \right) \right]}
\]  

(4.10)

### 4.1.2 Continuum Models

Many assumptions are implicit in the derivation of the continuum models investigated in this paper, but one is of particular importance. That is on a microscopic scale, blood can be treated as a homogeneous continuum. In other words, blood is statistically homogeneous over a sufficiently large sample volume, even through it is microscopically heterogeneous. Within such a volume one expects that a linear relation, analogous to Fick’s first law, will exist between the average diffusive flux and the average concentration gradient, where the average concentration is the volume fraction-weighted sum of the concentrations in RBCs and plasma, and the coefficient of proportionality is the effective diffusion coefficient.

#### 4.1.2.a Continuum Model (Oxyhemoglobin Augmentation)

This model (CMAug) treats blood as homogeneous hemoglobin (Hb) solution, taking into account the transport mechanism of molecular diffusion, convection and the facilitated diffusion of \(O_2\) due to the presence of Hb (Lemon et al., 1987):

\[
\frac{2Q}{\pi r_e^2} \left[ 1 - \left( \frac{r}{r_e} \right)^2 \right] \frac{\partial [O_2]}{\partial z} = \frac{D_{O_2}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]}{\partial r} \right) + C_{heme} \left[ j S - j' [O_2] \right] (1-S)
\]  

(4.11)

\[
\frac{2Q}{\pi r_e^2} \left[ 1 - \left( \frac{r}{r_c} \right)^2 \right] \frac{\partial S}{\partial z} = \frac{D_{HBO_2}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial S}{\partial r} \right) - [ j S - j' [O_2] ] (1-S)
\]  

(4.12)

where

- \(Q\) is the volumetric flowrate in the tube.
- \(C_{heme}\) is the total heme concentration in the suspension.
$j$ and $j'$ are the dissociation and association rate constants for O$_2$ binding to hemoglobin, respectively; $j$ is a constant, and $j'$ is a nonlinear function of [O$_2$].

The appropriate boundary conditions are

\[ @ z = 0, \quad [O_2] = [O_2]_{in}; \quad S = F_{eq}([O_2]_{in}) \]  
(4.13a)

\[ @ r = 0, \quad \frac{\partial [O_2]}{\partial r} = 0; \quad \frac{\partial S}{\partial r} = 0 \]  
(4.13b)

\[ @ r = r_r, \quad -D_{O2} \frac{\partial [O_2]}{\partial r} = \frac{K_{O2}}{\alpha_{O2} r_c \ln(GF)} ([O_2] - \alpha_{O2} P_{O2,ext}); \quad \frac{\partial S}{\partial r} = 0 \]  
(4.13c)

corresponding to uniform inlet concentrations, symmetry about centerline, and wall flux matching, respectively; and $\alpha_{O2}$ is the effective solubility coefficient for O$_2$ in blood.

4.1.2.b *Continuum Model (Local Chemical Equilibrium)*

This continuum model (CMequ) is slightly different from the previous one due to the fact that the augmentation of oxygen transport by diffusion of oxyhemoglobin is neglected (Reneau *et al.*, 1967; Buckles *et al.*, 1968; Weissman and Mockros, 1969; Bradley and Pike, 1971; Villarroel *et al.*, 1971; Benn *et al.*, 1975; Voorhees, 1976; Diller *et al.*, 1980). It assumes that the rate of reaction between O$_2$ and hemoglobin is sufficiently fast, when compared to the rate of diffusion of O$_2$ within the red cell, and that the reaction can be considered at local equilibrium. Therefore, the concentration of hemoglobin-bound O$_2$ is directly related to the concentration of dissolved O$_2$ via the oxyhemoglobin dissociation curve.

\[ \frac{2Q}{\pi r_c^2} \left[ 1 - \left( \frac{r}{r_c} \right)^2 \right] \left[ 1 + \frac{C_{heme}}{\alpha_{O2} \cdot dP_{O2}} \right] \frac{\partial [O_2]}{\partial z} = \frac{D_{O2}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]}{\partial r} \right) \]  
(4.14)
Equation (4.14) is solved subject to the appropriate inlet and boundary conditions similar to those that are imposed on Equation (4.11).

4.2 Numerical Analysis

4.2.1 Oxygen-Hemoglobin Equilibrium Relationship

Different investigators have used different approaches to handle the ODC. For instance, Diller et al. (1980) used a saturation curve which was based on the four-step Adair reaction scheme and developed by Margaria. If the Margaria approximation, Equation (4.15a), is chosen to describe the ODC; \( dS / dP_{O2} \) is then given by Equation (4.15b).

\[
S = \frac{(\kappa P_{O2})^3 + \eta - 1}{(\kappa P_{O2})^4 + \eta - 1} \quad (4.15a)
\]

\[
\frac{dS}{dP_{O2}} = \frac{(\kappa P_{O2})^6 + (\eta - 1)[4(\kappa P_{O2})^3 - 3(\kappa P_{O2})^2]}{k P_{O2}^2[(\kappa P_{O2})^4 + \eta - 1]^2} \quad (4.15b)
\]

where \( \kappa P_{O2} = \frac{1 + k P_{O2}}{k P_{O2}} \); \( k \) and \( \eta \) are constants.

Nair et al. (1990), however, chose the Hill equation to approximate the ODC (Equation (4.16a)), and the derivative is given by Equation (4.16b).

\[
S = \frac{P_{O2}^n}{1 + P_{O2}^n} \quad (4.16a)
\]

\[
\frac{dS}{dP_{O2}} = \frac{n (P_{O2})^n}{P_{O2} \left[1 + (P_{O2})^n \right]^2} \quad (4.16b)
\]
where $P_{50}$ and $n$ are the equilibrium parameters from experimental data.

There are other and slightly better approximations to the oxygen-hemoglobin equilibrium relationship, but those in general are more complicated (see Section 2.1.1.a). The selections here represent the simpler and reasonable choices; incorporation of both equilibrium approximations into the transport models results in similar predictions (differences of less than 1-2% in fractional saturation). Although equilibrium parameters from both kinetic models, $k$, $\eta$, $P_{50}$ and $n$, can be considered as useful indexes of the oxygen-carrying function of hemoglobin, $P_{50}$ and $n$ seem to reflect this property in a more direct fashion (they are directly obtainable from the ODC). Consequently, the Hill approximation was chosen for simulation of oxygen transport.

### 4.2.2 Parameter Values and Solutions of Transport Equations

The values for the parameters used in this study are listed in Table 4.1. For the continuum models, the effective physical properties (Brownian diffusion and solubility coefficients) are estimated by regarding the suspension as a hemolysate of the blood in the tube where the hemoglobin concentration is determined by the discharge hematocrit (solubility data from Christoforides and Hedley-Whyte (1969); diffusivity data from Kreuzer (1970), Keller et al.(1971) and Spaan et al.(1980)). However, the chemical environment is not altered as a result of this hypothetical hemolysis; thus equilibrium parameters of the blood; namely, $P_{50}$ and $n$, remain unchanged and are estimated using the oxygen saturation and partial pressure data obtained from the blood gas analyzer for the red cell suspensions. For the discrete model, parameters for the interior of the red cell are the same as those used and discussed in detail by Nair et al. (1988). In addition, parameters for the plasma region are needed: the diffusion coefficients comes from Kreuzer (1970) and the solubility coefficient from Christoforides and Hedley-Whyte (1969).
Table 4.1

Parameters used in the oxygen transport calculations.

<table>
<thead>
<tr>
<th>Discrete model parameters</th>
<th>Diller et al.</th>
<th>Schmukler and Chien</th>
<th>Boland et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{heme, rbc}}$ (mM)</td>
<td>20.8</td>
<td>20.8</td>
<td>20.8</td>
</tr>
<tr>
<td>$\left(\frac{\kappa}{\eta_{\text{rbc}}}\right)$ (μm⁻¹)</td>
<td>1.87</td>
<td>1.87</td>
<td>1.87</td>
</tr>
<tr>
<td>$\alpha_{O_2, \text{rbc}}$ (M/mm Hg)</td>
<td>1.43x10⁻⁶</td>
<td>1.43x10⁻⁶</td>
<td>1.43x10⁻⁶</td>
</tr>
<tr>
<td>$\alpha_{O_2, \text{pl}}$ (M/mm Hg)</td>
<td>1.27x10⁻⁶</td>
<td>1.33x10⁻⁶</td>
<td>1.33x10⁻⁶</td>
</tr>
<tr>
<td>$D_{O_2, \text{pl}}$ (cm²/sec)</td>
<td>2.25x10⁻⁵</td>
<td>2.70x10⁻⁵</td>
<td>2.70x10⁻⁵</td>
</tr>
<tr>
<td>$\delta$ (μm)</td>
<td>≈ 1.4</td>
<td>≈ 1.5</td>
<td>≈ 2.0</td>
</tr>
<tr>
<td>$B$ (blunting factor)</td>
<td>≈ 1.0</td>
<td>≈ 0.95</td>
<td>≈ 0.9</td>
</tr>
<tr>
<td>$H_T/H_D$</td>
<td>0.95 ($r_c=150 \mu m$)</td>
<td>0.94</td>
<td>0.83 (flowrate=12 μl/hr)</td>
</tr>
<tr>
<td> </td>
<td>1.00 ($r_c \geq 300 \mu m$)</td>
<td> </td>
<td>0.80 (flowrate=23 μl/hr)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Continuum model parameters</th>
<th>Diller et al.</th>
<th>Schmukler and Chien</th>
<th>Boland et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{heme}}$ (mM)</td>
<td>(calculated from $H_D$)</td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>$\alpha_{O_2}$ (M/mm Hg)</td>
<td>1.31x10⁻⁶ ($H_D=0.45$)</td>
<td>1.34x10⁻⁶</td>
<td>1.36x10⁻⁶ ($H_D=0.3$)</td>
</tr>
<tr>
<td> </td>
<td>1.30x10⁻⁶ ($H_D=0.24$)</td>
<td> </td>
<td>1.35x10⁻⁶ ($H_D=0.2$)</td>
</tr>
<tr>
<td>$D_{O_2}$ (cm²/sec)</td>
<td>1.65x10⁻⁵ ($H_D=0.45$)</td>
<td>2.55x10⁻⁵ ($H_D=0.1$)</td>
<td>2.30x10⁻⁵ ($H_D=0.3$)</td>
</tr>
<tr>
<td> </td>
<td>2.23x10⁻⁵ ($H_D=0.24$)</td>
<td>2.63x10⁻⁵ ($H_D=0.05$)</td>
<td>2.44x10⁻⁵ ($H_D=0.21$)</td>
</tr>
<tr>
<td> </td>
<td>2.68x10⁻⁵ ($H_D=0.015$)</td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>$D_{\text{HbO}_2}$ (cm²/sec)</td>
<td>3.80x10⁻⁷ ($H_D=0.45$)</td>
<td>≈ 11.9x10⁻⁷</td>
<td>7.60x10⁻⁷ ($H_D=0.3$)</td>
</tr>
<tr>
<td> </td>
<td>4.60x10⁻⁷ ($H_D=0.24$)</td>
<td> </td>
<td>9.00x10⁻⁷ ($H_D=0.2$)</td>
</tr>
</tbody>
</table>
Table 4.2
Summary of oxygen transfer models which are considered in Chapter 4.

<table>
<thead>
<tr>
<th>Model</th>
<th>Transport equation(s)</th>
<th>Dependent variable(s) calculated in the numerical solution</th>
<th>Equation(s) used to determine other dependent variable(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nair et al.'s discrete model</td>
<td>Equation (4.1) - Equation (4.4)</td>
<td>([O_2]<em>{rbc}(r,z)) and (S(r,z)) 0 ≤ (r) ≤ (r_r) [O_2]</em>{pl}(r,z) 0 ≤ (r) ≤ (r_r) ([O_2]_{pl}(r,z)) (r_r) ≤ (r) ≤ (r_c)</td>
<td>(S(r,z) = F_{eq}([O_2]<em>{rbc}(r,z))) 0 ≤ (r) ≤ (r_r) Equation (4.16a) ([O_2]</em>{pl}(r,z) = G([O_2]_{rbc}(r,z))) 0 ≤ (r) ≤ (r_c) Equation (4.17)</td>
</tr>
<tr>
<td>Simplified DM</td>
<td>Equation (4.8)</td>
<td>([O_2]_{rbc}(r,z)) 0 ≤ (r) ≤ (r_r)</td>
<td>(S(r,z) = F_{eq}([O_2]_{rbc}(r,z))) 0 ≤ (r) ≤ (r_r) Equation (4.16a)</td>
</tr>
<tr>
<td>CMaug</td>
<td>Equations (4.11) and (4.12)</td>
<td>(<a href="r,z">O_2</a>) and (S(r,z)) 0 ≤ (r) ≤ (r_c)</td>
<td>(S(r,z) = F_{eq}(<a href="r,z">O_2</a>)) 0 ≤ (r) ≤ (r_c) Equation (4.16a)</td>
</tr>
<tr>
<td>CMequ</td>
<td>Equation (4.14)</td>
<td>(<a href="r,z">O_2</a>) 0 ≤ (r) ≤ (r_c)</td>
<td>(S(r,z) = F_{eq}(<a href="r,z">O_2</a>)) 0 ≤ (r) ≤ (r_c) Equation (4.16a)</td>
</tr>
</tbody>
</table>
Solutions to these transport models are complicated by nonlinear kinetic terms, and in one case there is also coupling among the equations. Therefore, these equations are solved numerically using a finite element B-spline collocation method (described and evaluated elsewhere; Baxley and Hellums, 1983). The numerical solutions provide the spatial profiles of free and bound oxygen concentrations as functions of \( r \) and \( z \) for given values of various parameters (see Tables 4.1 and 4.2). In addition, for the discrete model, the dissolved \( O_2 \) concentration in the plasma phase can be obtained through Equation (4.17).

\[
\lbrack O_2 \rbrack_{pl} (r) = \left( \frac{\alpha_{O_2,pl}}{\alpha_{O_2, rbc}} \right) \lbrack O_2 \rbrack_{rbc} (r)
\]

(4.17a)

\[
\lbrack O_2 \rbrack_{pl} (r) = \left[ \frac{\alpha_{O_2,pl} P_{O_2,ext}}{\alpha_{O_2, rbc}} \lbrack O_2 \rbrack_{rbc} \bigg| r = rr \right] - \left( \frac{D_{O_2,pl} \alpha_{O_2,pl} ln(GF)}{K_{O_2}} + \frac{\alpha_{O_2,pl}}{\alpha_{O_2, rbc}} \lbrack O_2 \rbrack_{rbc} \bigg| r = rr \right) ln\left( \frac{r}{r_r} \right) + \frac{\alpha_{O_2,pl}}{\alpha_{O_2, rbc}} \lbrack O_2 \rbrack_{rbc} \bigg| r = rr
\]

(4.17b)

where \( \lbrack O_2 \rbrack_{pl} \) is the dissolved \( O_2 \) concentration in the RBC-free plasma region \((r_r < r < r_r)\).

### 4.2.3 Processing of Simulation Results for Comparison of Model Predictions with Experimental Measurements

#### 4.2.3.a Diller et al. (1980) and Schmukler and Chien (1985)

To compare theoretical predictions with experimental measurements obtained by Diller et al. (1980) and Schmukler and Chien (1985), the physically mixed mean concentrations of the blood at the tube outlet are required. It is first necessary to calculate the velocity-weighted bulk average values of dissolved oxygen concentration and oxyhemoglobin saturation. Then, the composition that would be measured if the
blood issuing from the tube were physically mixed and allowed to equilibrate can be calculated from the following relationship which pertains to the hemoglobin solutions:

\[ [O_2]_{mm} + C_{heme} S_{mm} = \overline{[O_2]} + C_{heme} \overline{S} \]  

(4.18)

where

\[ [O_2]_{mm} \text{ and } S_{mm} \] are the calculated overall mixed mean oxygen concentration and oxyhemoglobin saturation in the tube, respectively.

\[ \overline{[O_2]} \text{ and } \overline{S} \] are the physically mixed mean oxygen concentration and oxyhemoglobin saturation in the equilibrated sample, respectively.

Because enough time elapsed between collection of a sample and measurements on the sample via a blood gas analyzer to allow bulk equilibration of the entire RBC suspension; \( \overline{S} \) is related to \( \overline{[O_2]} \) via the equilibrium relationship; i.e., \( \overline{S} = F_{eq}(\overline{[O_2]}) \).

For the continuum models, if the Hill approximation is used, Equation (4.18) can be rewritten as the followings:

\[ [O_2]_{mm} + C_{heme} S_{mm} = \alpha_{O_2} \frac{\overline{P_{O_2}}}{P_{50}} + C_{heme} \left[ \frac{\overline{P_{O_2}}}{P_{50}} \right]^n \left[ 1 + \frac{\overline{P_{O_2}}}{P_{50}} \right]^n \]  

(4.19)

where \( \overline{P_{O_2}} \) is the equilibrated mixed mean oxygen tension in the collected sample. The mixed mean averages in Equation (4.19), \( [O_2]_{mm} \text{ and } S_{mm} \), are calculated as

\[ [O_2]_{mm}(z) = \frac{1}{\pi r_c^2} \int_0^{r_e} 4 \pi r \left[ 1 - \left( \frac{r}{r_c} \right)^2 \right] [O_2](r,z) \, dr \]  

(4.20a)
\[ S_{mm}(z) = \frac{1}{\pi r_c^2} \int_0^{r_c} 4 \pi r \left[ 1 - \left(\frac{r}{r_c}\right)^2 \right] y(r,z) \, dr \]  
(4.20b)

For the discrete model, Equation (4.18) can be rewritten as the following:

\[
\begin{align*}
[O_2]_{mm} + H_D \, C_{heme, rbc} \, S_{mm} &= H_D \alpha_{O_2, rbc} \, \overline{PO_2} \\
&+ (1 - H_D) \, \alpha_{O_2, pl} \, \overline{PO_2} + H_D \, C_{heme, rbc} \, \left[ \frac{\overline{PO_2}}{\overline{P_{50}}} \right]^n \\
&\left[ \frac{\overline{P_{50}}}{\overline{PO_2}} \right]^n \right] \]  
(4.21)
\end{align*}
\]

where

\( H_D \) is the discharge hematocrit, and \( H_D = \frac{1}{\pi r_c^2 \langle V \rangle} \int_0^{r_r} 2 \pi r \, h(r) \, V_{rbc}(r) \, dr \).

\( \langle V \rangle \) is the average velocity in the tube, and \( \langle V \rangle = Q / \pi r_c^2 \).

In this case, the mixed mean averages in Equation (4.21), \([O_2]_{mm}\) and \(S_{mm}\), are calculated as

\[
\begin{align*}
[O_2]_{mm}(z) &= \frac{1}{\pi r_c^2 \langle V \rangle} \int_0^{r_r} 2 \pi r \, h(r) \, V_{rbc}(r) \, [O_2]_{rbc}(r,z) \, dr \\
&+ \frac{1}{\pi r_c^2 \langle V \rangle} \int_0^{r_r} 2 \pi r \, (1 - h(r)) \, V_{pl}(r) \, [O_2]_{pl}(r,z) \, dr \\
&+ \frac{1}{\pi r_c^2 \langle V \rangle} \int_{r_r}^{r_c} 2 \pi r \, V_{pl}^*(r) \, [O_2]_{pl}^*(r,z) \, dr \]  
(4.22a)
\]

\[
S_{mm}(z) = \frac{1}{\pi r_c^2 H_D \langle V \rangle_{rbc}} \int_0^{r_c} 2 \pi r \, h(r) \, V_{rbc}(r) \, S(r,z) \, dr  
(4.22b)
\]
where \( \langle V \rangle_{rbc} \) is the average red cell velocity, and \( \langle V \rangle_{rbc} = Q H_D / \pi r_c^2 H_T \).

4.2.3.b Boland et al. (1987)

Boland et al.’s (1987) dual-wavelength microspectrophotometric system measures the space average oxygen saturation of the hemoglobin molecules, \( S_{sa} \), at various axial positions along the microvessel. To compare the simulation results with experimental data, the following operations are carried out:

For the continuum models,

\[
S_{sa}(z) = \frac{1}{\pi r_c^2} \int_{0}^{r_c} 2 \pi r S(r, z) \, dr
\]

(4.23)

For the discrete model,

\[
S_{sa}(z) = \frac{1}{\pi r_c^2 H_T} \int_{0}^{r_c} 2 \pi r h(r) S(r, z) \, dr
\]

(4.24)

The validity of Equation (4.24) is critically examined in Appendix A.

4.3 Results and Discussion

4.3.1 Simplified Discrete Model versus Nair et al.’s Model

A comparison is given of results from Nair et al.’s model and the simpler model (simplified DM) with experimental determinations from the artificial membrane tube system of Boland et al. (1987) which is described in Section 4.3.4. Equation (4.8) subject to the imposed inlet and boundary (Equation (4.10)) conditions was solved numerically by a finite element basis spline collocation method (described and evaluated
elsewhere; Baxley and Hellums, 1983). Figures 4.1 - 4.4 give the fractional saturation curves of hemoglobin, both $S_{sa}$ and $S_{nm}$, for release and uptake of O$_2$ comparing calculated results by Nair et al.'s model to those by the simplified model. The calculations were made for values of the parameters corresponding to specific experiments in the artificial membrane tube system, and the experimentally determined O$_2$ saturation values are given in the figures for comparison with the calculated values.

From these figures, it is clear that both models generate equally accurate predictions. The maximum deviation between the experimental data and the theoretical curve generated by either model is 0.1 fractional saturation units; the mean absolute deviation (calculated as $\frac{\sum_{i=1}^{N}|\text{deviation}_i|}{N}$ where $N$ is the number of experimental data points) and absolute algebraic deviation (calculated as $\frac{\left|\sum_{i=1}^{N}\text{deviation}_i\right|}{N}$) for any one curve does not exceed 0.05 and 0.04 fractional saturation units, respectively. A difference of no more than 0.03 in $S_{sa}$ and 0.02 in $S_{nm}$ are observed between the models for both the uptake and release cases. To put this small discrepancy into perspective, it is important to note that the difference between these models cannot be distinguished within the experimental error involved. Another observation is that the rate of change in O$_2$ saturation predicted by Nair et al.'s model is slightly lower than that predicted by the simplified model. This finding is consistent with the additional assumptions of the simplified model - some small transport resistances were neglected.

4.3.2 Comparison with Diller et al.'s Membrane Oxygenator Data (300 $\mu$m, 630 $\mu$m and 1000 $\mu$m in diameters)

Diller (1977) measured oxygenation of fresh human whole blood flowing through silicone-rubber membrane tubes with diameters ranging from 300 to 1000 $\mu$m. In this oxygen transfer apparatus, the membrane tube was divided into two by a
Figure 4.1: Comparison of models for $O_2$ release from RBC suspensions with a $H_2$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 µl/min in a 27-µm-diameter membrane tube at 37°C. The left panel gives the space average $O_2$ saturation ($S_{aO_2}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mO_2}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions, (---) simplified discrete model and (-----) Nair et al.’s model.
Figure 4.2: Comparison of models for O$_2$ uptake by RBC suspensions with a $H_D$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 μl/hr in a 27-μm-diameter membrane tube at 37 °C. The left panel gives the space average O$_2$ saturation ($S_{sc}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean O$_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions, (——) simplified discrete model and (- - - -) Nair et al.'s model.
Figure 4.3: Comparison of models for $O_2$ release from RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 27 mmHg flowing at 12 $\mu$L/hr in a 27-µm-diameter membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions, (——) simplified discrete model and (- - - -) Nair et al.'s model.
Figure 4.4: Comparison of models for O₂ uptake by RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 20 mmHg flowing at 23 μl/hr in a 27-μm-diameter membrane tube at 37 °C. The left panel gives the space average O₂ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean O₂ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions, (-----) simplified discrete model and (- - - -) Nair et al.’s model.
moveable plug. One part of the membrane tube served as the feed reservoir, and its environmental chamber was thus circulated with the same gas stream which was used to set the initial gas concentration of the fluid, \( P_{O2,\text{in}} \). The measured transfer, driven by the oxygen tension, \( P_{O2,\text{ext}} \), occurred in the other chamber. By moving the moveable plug, any of the ten different effective transfer lengths (10-100 cm) could be achieved for each sample. The permeability to oxygen of the medical grade silicone rubber is \( 3.1 \times 10^{-10} \text{ M cm}^2/\text{sec/mmHg} \) (Diller, 1977).

4.3.2.a Uptake Experiments with Saturated Blood

Figure 4.5 shows the results of the calculations obtained from both CMequ and simplified DM methods for simulating Diller's uptake experiments with saturated blood (runs S1 - S4). These experiments (\( P_{O2,\text{in}} > 100 \text{ mmHg} \) and \( P_{O2,\text{ext}} > 700 \text{ mmHg} \)) were designed to minimize the effect on transfer of the oxygen-hemoglobin reaction. Consequently, the hemoglobin saturation was maintained near 100%, and the value of \( dS/dP_{O2} = 0 \). Details on the experimental conditions of runs S1 - S4 are given in Table 4.3a. For oxygen saturated blood the continuum models, CMAug and CMequ, give the same predictions; so only one is shown. It can be seen that simplified DM generally predicts more transport than CMequ (for runs S1-S3, at \( z = 100 \text{ cm} \), DM predicts \( P_{O2} \) that is \( \approx 30 \text{ mmHg} \) that is higher than that predicted by CMequ); this is due to the fact that the value of \( D_{O2,\text{pl}} \) used (\( D_{O2,\text{pl}} = 2.25 \times 10^{-5} \text{ cm}^2/\text{sec} \); discrete model) is slightly higher than the value of \( D_{O2} \) (\( D_{O2} = 1.65 \times 10^{-5} \text{ cm}^2/\text{sec} \); homogeneous solution model). If \( V(r) = V_{\text{rbc}}(r) = V_p(r), \ h(r) = H_D \) and \( \alpha_{O2} = \alpha_{O2,\text{rbc}} = \alpha_{O2,\text{pl}} \), then simplified DM can be reduced to CMequ. Therefore, for large vessels where \( r_c \gg a, H_T/H_D = 1 \), the cell-depleted layer thickness is only a negligible fraction of the tube diameter, and the velocity profile is nearly parabolic, the difference between the simulation results obtained by simplified DM and CMequ can be accounted for by the differences between the values used for \( D_{O2,\text{pl}} \) and \( D_{O2} \); and between \( \alpha_{O2}, \alpha_{O2,\text{rbc}} \) and \( \alpha_{O2,\text{pl}} \). In run S4, a
### Table 4.3a

Experimental conditions for the saturated blood experiments (runs S1 - S4) conducted by Diller (1977).

<table>
<thead>
<tr>
<th>Run #</th>
<th>I.D. (µm)</th>
<th>O.D. (µm)</th>
<th>$Q$ (ml/min)</th>
<th>$H_D$</th>
<th>$P_{O_2,\text{in}}$ (mmHg)</th>
<th>$P_{O_2,\text{ext}}$ (mmHg)</th>
<th>$\gamma_{\text{avg}}$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>635</td>
<td>1219</td>
<td>3.76</td>
<td>0.44</td>
<td>105</td>
<td>711</td>
<td>1250</td>
</tr>
<tr>
<td>S2</td>
<td>1016</td>
<td>2158</td>
<td>1.90</td>
<td>0.40</td>
<td>120</td>
<td>710</td>
<td>150</td>
</tr>
<tr>
<td>S3</td>
<td>635</td>
<td>1219</td>
<td>3.29</td>
<td>0.41</td>
<td>130</td>
<td>715</td>
<td>1100</td>
</tr>
<tr>
<td>S4</td>
<td>1016</td>
<td>2158</td>
<td>3.29</td>
<td>0.24</td>
<td>148</td>
<td>708</td>
<td>270</td>
</tr>
</tbody>
</table>

### Table 4.3b

Experimental conditions for the unsaturated blood experiments (runs U1 - U6, US1 and US2) conducted by Diller (1977).

<table>
<thead>
<tr>
<th>Run #</th>
<th>I.D. (µm)</th>
<th>O.D. (µm)</th>
<th>$Q$ (ml/min)</th>
<th>pH</th>
<th>$P_{50}$</th>
<th>$H_D$</th>
<th>$P_{O_2,\text{in}}$ (mmHg)</th>
<th>$P_{O_2,\text{ext}}$ (mmHg)</th>
<th>$\gamma_{\text{avg}}$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3</td>
<td>305</td>
<td>635</td>
<td>0.376</td>
<td>7.30</td>
<td>28.0</td>
<td>0.44</td>
<td>47.4</td>
<td>120</td>
<td>119</td>
</tr>
<tr>
<td>U4</td>
<td>635</td>
<td>1219</td>
<td>0.376</td>
<td>7.35</td>
<td>28.0</td>
<td>0.42</td>
<td>23.3</td>
<td>119</td>
<td>118</td>
</tr>
<tr>
<td>U5</td>
<td>635</td>
<td>1219</td>
<td>0.329</td>
<td>7.35</td>
<td>27.0</td>
<td>0.42</td>
<td>30.3</td>
<td>118</td>
<td>119</td>
</tr>
<tr>
<td>U6</td>
<td>635</td>
<td>1219</td>
<td>0.329</td>
<td>7.30</td>
<td>27.0</td>
<td>0.45</td>
<td>42.0</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>US2</td>
<td>635</td>
<td>1219</td>
<td>0.329</td>
<td>7.40</td>
<td>28.0</td>
<td>0.42</td>
<td>24.7</td>
<td>712</td>
<td>116</td>
</tr>
<tr>
<td>U1</td>
<td>305</td>
<td>635</td>
<td>0.376</td>
<td>7.30</td>
<td>28.5</td>
<td>0.40</td>
<td>18.5</td>
<td>116</td>
<td>119</td>
</tr>
<tr>
<td>US1</td>
<td>635</td>
<td>1219</td>
<td>3.290</td>
<td>7.30</td>
<td>26.5</td>
<td>0.42</td>
<td>18.2</td>
<td>716</td>
<td>716</td>
</tr>
</tbody>
</table>
Figure 4.5: Comparison of models for oxygen uptake results for fully saturated fresh human blood flowing in silicone-rubber membrane tubes at 37 °C (details on the experimental conditions for runs S1 - S4 are given in Table 4.3a). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (——) simplified DM and (---) CMequ.
lower hematocrit \((H_D=24\%)\) sample was used; consequently, a higher value was estimated for \(D_{O2}\); and coincidentally, \(D_{O2}=D_{O2,pl}\) \((D_{O2}=2.23 \times 10^{-5} \text{ cm}^2/\text{sec})\). The maximum deviation between the theoretical curve generated by simplified DM and the experimental data is 33.5 mmHg units; the mean absolute deviation and absolute algebraic deviation for any one curve does not exceed 9.1 and 7.2 mmHg units, respectively. On the other hand, the maximum deviation between the theoretical curve generated by CMequ and the experimental data is 33.5 mmHg units; the mean absolute deviation and absolute algebraic deviation for any one curve does not exceed 18.6 and 18.6 mmHg units, respectively. Thus, it is seen that the simplified discrete model generates more accurate predictions than the continuum model; except for run S4 where both models give almost equivalent predictions.

4.3.2.b Uptake Experiments with Unsaturated Blood

Figures 4.6 - 4.12 show the experimental data on both the equilibrated mixed mean oxyhemoglobin saturation \((\bar{S})\) and oxygen tension \((P_{O2})\) in comparison to the predictions generated by both the continuum and discrete models. The oxygen transfer measurements are made with the blood initially at partial saturation \((P_{O2,in}=20-50 \text{ mmHg})\). Some of these experiments (runs U1 - U6) were designed to operate almost on the steep portion of the saturation curve \((P_{O2,ext}<120 \text{ mmHg})\); while other experiments (runs US1 and US2) were to operate over the full range of the curve \((P_{O2,ext}>700 \text{ mmHg})\). Details on the experimental conditions of these runs are given in Tables 4.3b and 4.4.

U3 - U6 and US2: runs where shear-induced augmentation of oxygen is negligible

Molecular diffusivity was used in these simulations; with fresh human blood at 37 \(^\circ\text{C}\) and \(H_D=40\%\), for the continuum models: \(D_{O2}=1.65 \times 10^{-5} \text{ cm}^2/\text{sec}\) and \(D_{HbO2}=3.8 \times 10^{-7} \text{ cm}^2/\text{sec}\), for the discrete model: \(D_{O2,pl}=2.25 \times 10^{-5} \text{ cm}^2/\text{sec}\) (see Table 4.1). From Figures 4.6 - 4.10, it is seen that CMaug consistently predicts more
Figure 4.6: Comparison of models for oxygen uptake by fresh human blood with a $H_D$ of 44% and $P_{50}$ of 28 mmHg flowing at 0.376 ml/min in a 305-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions for run U3 is given in Table 4.3b). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean $O_2$ partial pressure ($P_{O_2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (——) simplified DM, (---) CMequ and (— —) CMaug.
Figure 4.7: Comparison of models for oxygen uptake by fresh human blood with a $H_D$ of 42% and $P_{50}$ of 28 mmHg flowing at 0.376 ml/min in a 635-µm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions for run U4 is given in Table 4.3b). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean $O_2$ partial pressure ($\bar{P}_{O_2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (---) simplified DM, (- - - -) CMequ and (---) CMaug.
Figure 4.8: Comparison of models for oxygen uptake by fresh human blood with a $P_{O_2}$ of 42% and $P_{CO_2}$ of 27 mmHg flowing at 0.329 ml/min in a 635-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions for run U5 is given in Table 4.3b). The left panel gives the equilibrated mixed mean oxygen saturation ($\bar{S}_O_2$) at different axial positions along the membrane oxygenator; the right panel, the equilibrated mixed mean $O_2$ partial pressure ($P_{O_2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (-----) simplified DM, (--.-) CMequ and (- - - -) CMaug.
Figure 4.9: Comparison of models for oxygen uptake by fresh human blood with a $H_D$ of 45% and $P_{50}$ of 27 mmHg flowing at 0.329 ml/min in a 635-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions for run U6 is given in Table 4.3b). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean O$_2$ partial pressure ($P_{O2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (-- -- --) simplified DM, (- - - -) CMequ and (--- --) CMaug.
Figure 4.10: Comparison of models for oxygen uptake by fresh human blood with a $H_D$ of 42% and $P_{50}$ of 28 mmHg flowing at 0.329 ml/min in a 635-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions for run US2 is given in Table 4.3b). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($S$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean $O_2$ partial pressure ($P_{O2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation curves for the same conditions; (— — —) simplified DM, (— - - - ) CMequ and (— - — — ) CMaug.
transport than the other two models. However, upon setting the value of \( D_{HbO2} \) zero, the calculations generated by CMaug and CMequ become identical. In addition, both simplified DM and CMequ generate accurate predictions which agree with the measured values (equilibrated mixed mean oxygen saturation and tension) for runs U3 - U6 and US2 to well within the experimental error. Here simplified DM approach predicts slightly more transport than CMequ; this is, again, due to the values used for \( D_{O2,pl} \) and \( D_{O2} \). For runs U3 - U6 and US2 differences of no more than 0.35 mM in \( [O_2]_{total} \), total \( O_2 \) content, were observed between simulation results generated by DM and CMaug; and differences of no more than 0.35 mM in \( [O_2]_{total} \) were observed between the DM and CMequ. The maximum deviations between the theoretical curves generated by simplified DM, CMaug and CMequ from the experimental data are 0.24 mM, 0.41 mM, and 0.54 mM in \( [O_2]_{total} \), respectively. The algebraic deviations for any one curve generated by simplified DM, CMaug and CMequ from the experimental data do not exceed 0.10 mM, 0.21 mM and 0.32 mM in \( [O_2]_{total} \), respectively.

Table 4.4: Values of local and overall operating slopes of ODC and average shear rate for the unsaturated blood experiments conducted by Diller (1977).

<table>
<thead>
<tr>
<th>Run #</th>
<th>( m = \frac{H_D Ch_{ene}}{\alpha_{O2}} \frac{dS_{O2}}{dP_{O2}} )</th>
<th>( M = \frac{H_D Ch_{ene, rbc}}{\alpha_{O2}} )</th>
<th>( \frac{S_{P_{O2}=P_{O2,eu}} - S_{P_{O2}=P_{O2,in}}}{P_{O2,eu} - P_{O2,in}} )</th>
<th>( \gamma_{avg} ) (sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3</td>
<td>42</td>
<td>18.9</td>
<td></td>
<td>985</td>
</tr>
<tr>
<td>U4</td>
<td>138</td>
<td>41.5</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>U5</td>
<td>124</td>
<td>30.8</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>U6</td>
<td>44</td>
<td>20.5</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>US2</td>
<td>( m_1 = 158 ) ( m_2 = 70 )</td>
<td>5.4</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>U1</td>
<td>154</td>
<td>46.3</td>
<td></td>
<td>1125</td>
</tr>
<tr>
<td>US1</td>
<td>150</td>
<td>6.8</td>
<td></td>
<td>1095</td>
</tr>
</tbody>
</table>

\( ^\dagger \) \( m_1 \) and \( m_2 \) for run US2 in Table 4.4 represent the local operating slopes along the unsaturated and almost saturated portions of the ODC, respectively.
U1 and US1: runs where shear-induced augmentation of oxygen is important

Figures 4.11 and 4.12 give the comparison of the results computed by CMequ using different values of effective diffusivity for $O_2$, $D_{O2,eff}$, with the experimental data from runs U1 and US1. The bottom curve in both plots is generated using ordinary diffusivities ($D_{O2}=1.65 \times 10^{-5}$ cm$^2$/sec and $D_{O2,pt}=2.25 \times 10^{-5}$ cm$^2$/sec). It is clear that without the usage of the effective diffusivity which includes the contribution from shear-induced augmentation, CMequ under estimates the amount of transport. The uppermost curve is generated using effective diffusivity estimated from Equations (2.19a) and (2.19b); the intermediate set is generated using Equations (2.19a) and (2.19b) with $m$, the local operating slope, being replaced with $M$, the overall operating slope. The effective RBC diffusion coefficient, $D_{rbc}$, is evaluated from Zydneb and Colton’s correlation (1988). Using the equivalent spherical radius of red cell, $a=2.75$ μm, and whole blood hematocrit, $H_D=0.4$, $D_{rbc}$ is calculated to be $(3 \times 10^{-9}$ cm$^2$) ($\gamma_{avg}$ sec$^{-1}$) from Equation (2.16). The effective oxygen diffusivity in the suspension is then the sum of the ordinary diffusivity and the shear-induced diffusivity. If one were to use $m$ to estimate the effective diffusivity; the models end up over predicting the transport observed in both runs U1 and US1. Better agreements between the model simulations and experimental measurements were achieved by using $M$ in place of $m$ (see Figures 4.11 and 4.12). Figure 4.13 provides a comparison between simplified DM and CMequ under the experimental condition of U1 and using both $D_{O2,eff}=D_{O2, SF}$ and $D_{O2,eff}=D_{O2, SF}+D_{rbc}(1+M)$. It is clear from this plot that the differences in the results generated by the two models are small for both sets of diffusivity values. In addition, for both models the usage of a modified diffusivity, $D_{O2,eff}=D_{O2, SF}+D_{rbc}(1+M)$, to also include the shear-induced augmentation of $O_2$ provides a better agreement between the simulation and experimental results.
Figure 4.11: Oxygen uptake result by fresh human blood with a $H_D$ of 40% and $P_{50}$ of 28.5 mmHg flowing at 0.376 ml/min in a 305-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions of run U1 is given in Tables 4.3b and 4.4). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($S$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean $O_2$ partial pressure ($P_{O_2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation of experiments generated by CMequ: the bottom curve (-----) is generated using $D_{O_2,eff}=1.65\times10^{-5}$ cm$^2$/sec; the intermediate curve (- - - -) is generated using $D_{O_2,eff}=17.6\times10^{-5}$ cm$^2$/sec; the uppermost curve (------) is generated using $D_{O_2,eff}=54.0\times10^{-5}$ cm$^2$/sec.
Figure 4.12: Oxygen uptake result by fresh human blood with a $H_D$ of 42% and $P_{SO}$ of 26.5 mmHg flowing at 3.29 ml/min in a 635-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental conditions of run US1 is given in Tables 4.3b and 4.4). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean O$_2$ partial pressure ($P_{O2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation of experiments generated by CMequ: the bottom curve (-----) is generated using $D_{O2,\text{eff}}=1.65 \times 10^{-5}$ cm$^2$/sec; the intermediate curve (--- - - -) is generated using $D_{O2,\text{eff}}=4.21 \times 10^{-5}$ cm$^2$/sec; the uppermost curve (-----) is generated using $D_{O2,\text{eff}}=51.2 \times 10^{-5}$ cm$^2$/sec.
Figure 4.13: Comparison of oxygen uptake result by fresh human blood with a $H_D$ of 40% and $P_{50}$ of 28.5 mmHg flowing at 0.376 ml/min in a 305-μm-diameter silicone-rubber membrane tube at 37 °C (more detail on the experimental condition of run U1 is given in Tables 4.3b and 4.4). The left panel gives the equilibrated mixed mean oxyhemoglobin saturation ($S$) at different axial positions along the membrane oxygenator; and the right panel, the equilibrated mixed mean $O_2$ partial pressure ($P_{O_2}$). Circles: experimental data from Diller (1977). Curves: theoretical simulation of experiments; (——) simplified DM and (- - - - ) CMequ. The bottom set of curves is generated using $D_{O_2, eff} = 2.25 \times 10^{-5}$ cm$^2$/sec for simplified DM and $D_{O_2, eff} = 1.65 \times 10^{-5}$ cm$^2$/sec for CMequ; the upper set of curves is generated using $D_{O_2, eff} = 18.2 \times 10^{-5}$ cm$^2$/sec for simplified DM and $D_{O_2, eff} = 17.6 \times 10^{-5}$ cm$^2$/sec for CMequ.
4.3.2.c  Analysis of Enhanced Transfer

The values for $m$, $M$ and $\gamma_{avg}$ for various runs are listed in Table 4.4. The local operating slopes for these runs are approximately constant, except for run US2 in which the blood experiences both the unsaturated and almost saturated regions of the ODC ($m_1$ and $m_2$ for run US2 in Table 4.4 represent the local operating slopes in the unsaturated and almost saturated portions of the dissociation curve, respectively). From Table 4.4, it is seen that runs U1 and US1 were operated both along the steep portion of the ODC ($m>150$) and under high shear rate environment ($\gamma_{avg}>1000$ sec$^{-1}$); while other runs were operated similarly with high oxygen sink strength but at low shear rate (runs U4, U5 and US2), or low oxygen carriage potential but at high shear rate (run U3), or low $m$ and low shear rate (run U6). The result indicates that shear-dependent augmentation is not significant in saturated whole blood flowing through an oxygenated environment. The presence of an oxygen sink inside the red cells would provide an additional mechanism of augmented transport only if high shear rate is also available in the flow field. From the simple analysis outlined earlier in Section 2.2.2, it is seen that the magnitude of the augmentation due to the lateral migration of unsaturated RBCs in the radial direction, which could be potentially large, is theoretically related to the product of $D_{rbc}m$. However, from analyzing Diller's experimental results on runs U1 and US1, the outcome seems to demonstrate that the values of $m$ along with $\gamma$ can be used as indicators for monitoring whether the effect of shear-induced augmentation is substantial or not; but the magnitude of the augmentation is related to $D_{rbc}M$. Tables 4.5 and 4.6 summarize the difference between Diller et al.'s experimental results and the calculations generated by simplified DM and CMequ using three different values of $D_{O2,eff}$.

These results illustrate that for vessels with diameters of 300 $\mu$m and larger augmented $O_2$ transport is significant only under the conditions of both high $m$ and $\gamma$. In addition, the magnitude of this effect was shown theoretically to be related to the
Table 4.5

Comparison of Diller's oxygen transfer data to the simulation results generated by simplified DM using three different values of $D_{O2,\text{eff}}$. Both the maximum deviation and algebraic deviation\( ^\dagger \) of the numerical calculations from the experimental data are given here for $[O_2]_{\text{tot}}$, the total O_2 content.

<table>
<thead>
<tr>
<th>$D_{O2,\text{eff}}$</th>
<th>$D_{O2,\text{eff}}=D_{O2,\text{SF}}$</th>
<th>$D_{O2,\text{eff}}=D_{O2,\text{SF}}+D_{\text{rbc}}(1+M)$</th>
<th>$D_{O2,\text{eff}}=D_{O2,\text{SF}}+D_{\text{rbc}}(1+m)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error</td>
<td>lmax. deviation</td>
<td>algebraic deviation</td>
<td>lmax. deviation</td>
</tr>
<tr>
<td>Run U3</td>
<td>0.057 mM</td>
<td>0.001 mM ↓(^*)</td>
<td>0.512 mM</td>
</tr>
<tr>
<td>Run U4</td>
<td>0.154 mM</td>
<td>0.005 mM ↑(\uparrow)</td>
<td>0.352 mM</td>
</tr>
<tr>
<td>Run U5</td>
<td>0.071 mM</td>
<td>0.025 mM ↓(\downarrow)</td>
<td>0.083 mM</td>
</tr>
<tr>
<td>Run U6</td>
<td>0.032 mM</td>
<td>0.001 mM ↓(\downarrow)</td>
<td>0.101 mM</td>
</tr>
<tr>
<td>Run US2</td>
<td>0.242 mM</td>
<td>0.108 mM ↓(\downarrow)</td>
<td>0.189 mM</td>
</tr>
<tr>
<td>Run U1</td>
<td>1.09 mM</td>
<td>0.644 mM ↓(\downarrow)</td>
<td>0.326 mM</td>
</tr>
<tr>
<td>Run US1</td>
<td>0.351 mM</td>
<td>0.191 mM ↓(\downarrow)</td>
<td>0.212 mM</td>
</tr>
</tbody>
</table>

\(\dagger\) The algebraic derivation is calculated as $\sum_{i=1}^{N} (\text{deviation})/N$ where $N$ is the number of data points.

\(^*\) ↓\(\downarrow\) indicates that a model on the average under predicts the amount of transport, and ↑\(\uparrow\) indicates that a model on the average over predicts the amount of transport.
Table 4.6

Comparison of Diller’s oxygen transfer data to the simulation results generated by CMequ using three different values of $D_{O_2,eff}$. Both the maximum deviation and algebraic deviation\(^*\) of the numerical calculations from the experimental data are given here for $[O_2]_{total}$, the total O\(_2\) content.

<table>
<thead>
<tr>
<th>$D_{O_2,eff}$</th>
<th>$D_{O_2}=D_{O_2, SF}$</th>
<th>$D_{O_2}=D_{O_2, SF}+D_{rbc}(1+M)$</th>
<th>$D_{O_2}=D_{O_2, SF}+D_{rbc}(1+m)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error</td>
<td>lmax. deviation</td>
<td>algebraic deviation</td>
<td>lmax. deviation</td>
</tr>
<tr>
<td>Run U3</td>
<td>0.037 mM</td>
<td>0.035 mM ↓(^*)</td>
<td>0.520 mM</td>
</tr>
<tr>
<td>Run U4</td>
<td>0.296 mM</td>
<td>0.018 mM ↑</td>
<td>0.288 mM</td>
</tr>
<tr>
<td>Run U5</td>
<td>0.162 mM</td>
<td>0.086 mM ↓</td>
<td>0.094 mM</td>
</tr>
<tr>
<td>Run U6</td>
<td>0.116 mM</td>
<td>0.061 mM ↓</td>
<td>0.067 mM</td>
</tr>
<tr>
<td>Run US2</td>
<td>0.544 mM</td>
<td>0.318 mM ↓</td>
<td>0.383 mM</td>
</tr>
<tr>
<td>Run U1</td>
<td>1.17 mM</td>
<td>0.704 mM ↓</td>
<td>0.386 mM</td>
</tr>
<tr>
<td>Run US1</td>
<td>0.403 mM</td>
<td>0.216 mM ↓</td>
<td>0.045 mM</td>
</tr>
</tbody>
</table>

\(^*\) The algebraic derivation is calculated as $\sum_{i=1}^{N} (\text{deviation})_i / N$ where $N$ is the number of data points.

\(*\) ↓ indicates that a model on the average under predicts the amount of transport, and ↑ indicates that a model on the average over predicts the amount of transport.
magnitude of $D_{rbc}m$; however, Diller et al.'s experimental results seem to point toward $D_{rbc}M$. As a result, more experimental data is needed for further investigation and quantification of this phenomena as a function of vessel diameter, oxygen storage potential ($m$ or $M$) and shear rate. Furthermore, it should be mentioned that for runs U1 and US1 implementation of Equation (2.20), which was developed by Diller and Mikic (1983) for estimating $D_{O2,eff}$, in the O$_2$ transport models generated results which were similar to that predicted by using Equation (2.19) with $m$ being replaced with $M$. Equation (2.19) is on a theoretically more sound basis in the sense that Equation (2.20) does not include the effect of dispersive fluid migration which is proportional to $(1-H_D)D_{rbc}$. The effect of dispersive fluid migration is minor when $M >> 1$, but it could become important under the conditions of small $M$ and high $\gamma$.

4.3.3 Comparison with Schmukler and Chien's Membrane Deoxygenator Data (100 $\mu$m in diameter)

Schmukler and Chien (1985) measured deoxygenation of red cells flowing through 100-μm-diameter microporous polypropylene hollow fibers. The deoxygenators were composed of fiber beds consisting of 96 or 144 parallel fibers and with 5 or 10 cm active length. The incoming sample to the deoxygenator was preoxygenated to give a $P_{O2, in} = 91$ mmHg. A stream of humidified 95% nitrogen - 5% carbon dioxide ($P_{O2, ext} = 0$ mmHg) flowed counter-currently in the environmental chamber. The outgoing sample from the deoxygenator was directly connected to a blood gas analyzer, and time was allowed for equilibration before readings were obtained. The permeability of the polypropylene hollow fibers used in the deoxygenator was obtained by analyzing the authors' results on hemoglobin solution, and it was determined to be $1 \times 10^{-11} \text{Mcm}^2/\text{sec/mmHg}$ (Boland et al., 1987). The $P_{50}$ of the blood was taken to be 27 mmHg as typical of red cell suspensions at the experimental $pH$ of
7.4. All other parameters required for the theoretical simulation were specified by Schmukler and Chien.

Figure 4.14 shows the results of the calculations generated by the models corresponding to both the outgoing equilibrated mixed mean oxygen tension and saturation as functions of the residence time of the red cell suspension. The residence time in the deoxygenator is defined as the ratio of the active fiber length to the average fluid velocity in the tube. From both plots, it is clear that the differences in simulation results for these models are small. Differences of no more than 2.2 mmHg in oxygen tension and 0.03 in fractional saturation of hemoglobin were observed between the results from the simplified DM and CMAug; and differences of no more than 0.5 mmHg in oxygen tension and 0.01 in saturation of hemoglobin between the simplified DM and CMequ. Furthermore, the agreement between the theoretical curves and the experimental data is excellent. In the worst case, the hematocrit of 1.5%, the maximum deviations and the algebraic average deviations between the theoretical results generated by simplified DM, CMAug, CMequ and the experimental data are 7.7, 7.2, 6.7 and 3.4, 3.0, 2.3 mmHg units, respectively.

It has been reported that in dilute suspensions particle rotation augments the mass transport (Keller, 1971; Wang and Keller, 1985). However, this effect was found to be insignificant in analyzing Schmukler and Chien’s data. As illustrated in Figures 4.15 - 4.16, usage of the ordinary molecular diffusivity is sufficient for predicting the observed transport phenomena. Introduction of shear-induced diffusivity into the models leads to over prediction of the transport for the \( H_D=10\% \) and 5% cases. Table 4.7 summarizes the maximum deviation and algebraic average deviation of the numerical results generated by simplified DM and CMequ using three different values of effective diffusivity for \( O_2 \) from the experimental data.
Figure 4.14: Comparison of models for oxygen release from red cell suspensions with $P_{50}$ of 27 mmHg flowing in microporous polypropylene hollow fibers with 100 μm in I.D. and 130 μm in O.D. at 37 °C. The left panel gives the equilibrated mixed mean $O_2$ partial pressure ($\bar{P}_{O_2}$) as a function of residence time; and the right panel, the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$). Data points: means of experiments from Schmukler and Chien (1985). Curves: theoretical simulation curves for the same conditions; (——) simplified DM, (-----) CMequ and (— — —) CMaug.
Figure 4.15: Oxygen release from red cell suspensions with a $H_D$ of 10% and $P_{50}$ of 27 mmHg flowing in microporous polypropylene hollow fibers with 100 μm in I.D. and 130 μm in O.D. at 37 °C. The left panel gives the equilibrated mixed mean $O_2$ partial pressure ($P_{O2}$) as a function of residence time; and the right panel, the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$). Data points: means of experiments from Schmukler and Chien (1985). Curves: theoretical simulation of experiments generated by CMequ: the upper curve (——) is generated using $D_{O2,eff}=1.65\times10^{-5}$ cm$^2$/sec; the intermediate curve (-----) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+M)$ where $M=16.5$; the bottom curve (— — —) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b).
Figure 4.16: Oxygen release from red cell suspensions with a $H_D$ of 5% and $P_{50}$ of 27 mmHg flowing in microporous polypropylene hollow fibers with 100 µm in I.D. and 130 µm in O.D. at 37 °C. The left panel gives the equilibrated mixed mean $O_2$ partial pressure ($\bar{P}_O_2$) as a function of residence time; and the right panel, the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$). Data points: means of experiments from Schmukler and Chien (1985). Curves: theoretical simulation of the experiments generated by CMequ: the upper curve (-----) is generated using $D_{O_2, eff}=1.65\times10^{-5}$ cm²/sec; the intermediate curve (- - - -) is generated using $D_{O_2, eff}=D_{O_2, SF}+D_{rbc}(1+M)$ where $M=8.3$; the bottom curve (--- ---) is generated using $D_{O_2, eff}=D_{O_2, SF}+D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b).
Figure 4.17: Oxygen release from red cell suspensions with a $H_D$ of 1.5% and $P_{50}$ of 27 mmHg flowing in microporous polypropylene hollow fibers with 100 μm in I.D. and 130 μm in O.D. at 37 °C. The left panel gives the equilibrated mixed mean $O_2$ partial pressure ($P_{O2}$) as a function of residence time; and the right panel, the equilibrated mixed mean oxyhemoglobin saturation ($\bar{S}$). Data points: means of experiments from Schmukler and Chien (1985). Curves: theoretical simulation of experiments generated by CMequ: the upper curve ($\cdots$) is generated using $D_{O2,eff}=1.65 \times 10^{-5}$ cm$^2$/sec; the intermediate curve (- - - -) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+M)$ where $M=2.5$; the bottom curve (— - —) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b).
Table 4.7

Comparison of Chien and Schmukler's oxygen transfer data to the simulation results generated by simplified DM and CMequ using three different values of $D_{O2, eff}$. Both the maximum deviation and algebraic deviation of the numerical calculations from the experimental data are given here for $P_{O2}$, the equilibrated mixed mean outgoing $P_{O2}$.

<table>
<thead>
<tr>
<th>Error</th>
<th>$D_{O2, eff}=D_{O2, SF}$</th>
<th>$D_{O2, eff}=D_{O2, SF}+D_{rbc}(1+M)$</th>
<th>$D_{O2, eff}=D_{O2, SF}+D_{rbc}(1+m)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_D=10%$</td>
<td>2.5 mmHg 0.79 mmHg $\downarrow*$</td>
<td>2.9 mmHg 1.2 mmHg $\uparrow^*$</td>
<td>4.9 mmHg 4.1 mmHg $\uparrow$</td>
</tr>
<tr>
<td>$H_D=5%$</td>
<td>1.2 mmHg 0.48 mmHg $\downarrow$</td>
<td>1.5 mmHg 0.57 mmHg $\uparrow$</td>
<td>3.3 mmHg 2.0 mmHg $\uparrow$</td>
</tr>
<tr>
<td>$H_D=1.5%$</td>
<td>7.7 mmHg 3.4 mmHg $\downarrow$</td>
<td>7.1 mmHg 3.2 mmHg $\downarrow$</td>
<td>6.9 mmHg 2.8 mmHg $\downarrow$</td>
</tr>
</tbody>
</table>

Continuum model: CMequ

<table>
<thead>
<tr>
<th>Error</th>
<th>$D_{O2}=D_{O2, SF}$</th>
<th>$D_{O2}=D_{O2, SF}+D_{rbc}(1+M)$</th>
<th>$D_{O2}=D_{O2, SF}+D_{rbc}(1+m)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_D=10%$</td>
<td>2.2 mmHg 0.48 mmHg $\downarrow$</td>
<td>3.1 mmHg 1.8 mmHg $\uparrow$</td>
<td>9.4 mmHg 7.0 mmHg $\uparrow$</td>
</tr>
<tr>
<td>$H_D=5%$</td>
<td>1.9 mmHg 0.50 mmHg $\downarrow$</td>
<td>2.5 mmHg 1.2 mmHg $\uparrow$</td>
<td>5.4 mmHg 3.7 mmHg $\uparrow$</td>
</tr>
<tr>
<td>$H_D=1.5%$</td>
<td>7.2 mmHg 3.0 mmHg $\downarrow$</td>
<td>6.4 mmHg 2.7 mmHg $\downarrow$</td>
<td>6.0 mmHg 2.1 mmHg $\downarrow$</td>
</tr>
</tbody>
</table>

$* \downarrow$ indicates that a model on the average under predicts the amount of transport, and $\uparrow$ indicates that a model on the average over predicts the amount of transport.
4.3.4 Comparison with Boland et al.'s Artificial Membrane Tube Data (27 \( \mu \)m in diameter)

Another comparison was performed by utilizing data obtained by Boland et al. for red cell suspensions flowing in a 27-\( \mu \)m-diameter and 5-mm-active length silicone rubber capillary which is embedded in a 170-\( \mu \)m-thickness slab. The permeability of the silicone copolymer is 4.17x10\(^{-10}\) M cm\(^2\)/sec/mmHg (Boland et al., 1987; Nair et al., 1989). Oxygen uptake experiments were conducted by suffusing the gas space surrounding the gas space surrounding the capillary with oxygen-nitrogen mixture \((P_{O2, ext}=159.6 \text{ mmHg})\) and perfusing the capillary with deoxygenated samples \((P_{O2, in}=0 \text{ mmHg})\). Oxygen release experiments were carried out by suffusing the gas space with pure nitrogen \((P_{O2, ext}=0 \text{ mmHg})\) and perfusing the capillary with deoxygenated samples \((P_{O2, in}=159.6 \text{ mmHg})\). Experimental determinations were obtained over a rectangular field of view of approximately 28x40 \( \mu \)m with a dual-wavelength microspectrophotometer to yield space-averaged fractional saturations of hemoglobin at different axial positions along the microvessel.

Figures 4.18 - 4.21 show both the space and mixed mean averages of oxygen saturation as functions of axial position; results are shown for both release and uptake for several values of the parameters \(P_{50}, H_D\) and \(Q\). The left-hand-side panels of these plots which present the comparison of the experimentally obtained space-averaged oxygen saturation of hemoglobin to the predictions generated by various models indicate that simplified DM gives better agreement than the continuum models. Both continuum models, CMaug and CMequ, underestimate the resistance to oxygen transport and thus over predict the amount of transport that occurs in the small microvessels. It is also observed that the continuum model, CMequ, which assumes local chemical equilibrium and thus neglects the facilitated diffusion due to oxyhemoglobin predicts a higher resistance and results in a closer fit to the experimental data. A more appropriate means
Figure 4.18: Comparison of models for oxygen release from RBC suspensions with a $H_D$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 µl/hr in a 27 µm-diameter-membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions; (-----) simplified DM, (- - - -) CMequ and (— — —) CMaug.
Figure 4.19: Comparison of models for oxygen uptake by RBC suspensions with a $H_D$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 μl/hr in a 27-μm-diameter membrane tube at 37 °C. The left panel gives the space average O₂ saturation ($S_{\text{soc}}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean O₂ saturation ($S_{\text{mm}}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions; (— — — ) simplified DM, (-----) CMequ and (— . —) CMaug.
Figure 4.20: Comparison of models for oxygen release from RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 27 mmHg flowing at 12 µl/hr in a 27-µm-diameter membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sg}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions; (———) simplified DM, (---...) CMequ and (—..—) CMaug.
Figure 4.21: Comparison of models for oxygen uptake by RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 20 mmHg flowing at 23 µl/hr in a 27-µm-diameter membrane tube at 37 °C. The left panel gives the space average O$_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean O$_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation curves for the same conditions; (———) simplified DM, (- - - -) CMequ and (— - -) CMaug.
of comparing these models is to compare the calculated mixed mean concentrations (the right-hand-side panels of Figures 4.18 - 4.21). It is observed that the difference between DM and CMequ on this basis is smaller. There is a difference of no more than 0.1 in $S_{nm}$ which would be within the experimental error involved. This seems to indicate that CMequ is satisfactory for predicting oxygen transport in a 27-μm-diameter microvessel, although it slightly over predicts the amount of transport.

Figures 4.22 and 4.23 give plots of the internal (calculated) radial profiles of hemoglobin oxygen saturation at several axial positions for both release and uptake cases. Comparing the detailed oxygen saturation profiles for these models, it can be seen that the profiles generated by simplified DM and CMequ are in reasonable agreement except at the cell-depleted region. CMaug predicts notably more transport than DM across almost the entire tube, except for the region near the tube wall. The space average receives a larger contribution from the blood travelling near the wall; while on the contrary, the mixed mean weights blood in the center of the tube more heavily. Consequently, when the models are judged based on the mixing-cup averages, CMequ gives similar predictions as DM, but CMaug performs poorly; while if the determining criteria is space-averaged saturations, both CMequ and CMaug predict significantly more transport than DM does.

Although the hemoglobin in these runs operated mostly over the steep portion of the ODC ($m_1=120$ and $m_2=30; M \approx 20$) and at average shear rates of about 800 to 1500 sec$^{-1}$, shear-induced enhancement of oxygen transfer to and from the blood was not observed. As illustrated in Figures 4.24 - 4.27, with the usage of molecular diffusivity the simplified DM accurately predicts the transfer rate. On the other hand, upon incorporation of an effective diffusivity to account for the the possible shear-induced augmentation of $O_2$ transport, the model generates results which are substantially in error (see Table 4.8). This is not surprising because it is known that red cell rotation
**Figure 4.22:** Comparison of the internal (nonmeasurable) profiles of oxyhemoglobin saturation as function of axial distance and dimensionless radial position which are generated by simplified DM, CMequ and CMaug for the experimental conditions of R1. (---) $z = 0$ mm; (---) $z = 1$ mm; (---) $z = 2$ mm; (---) $z = 3$ mm; (---) $z = 4$ mm; (---) $z = 5$ mm.
Figure 4.23: Comparison of the internal (nonmeasurable) profiles of oxyhemoglobin saturation as function of axial distance and dimensionless radial position which are generated by simplified DM, CMequ and CMaug for the experimental conditions of U1. (—○—) z = 0 mm; (—□—) z = 1 mm; (—■—) z = 2 mm; (—■—) z = 3 mm; (—•—) z = 4 mm; (—Δ—) z = 5 mm.
Figure 4.24: Oxygen release from RBC suspensions with a $H_D$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 $\mu$L/hr in a 27-$\mu$m-diameter membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation of experiments generated by simplified DM: the upper curve (---------) is generated using $D_{O_2, eff} = 2.25 \times 10^{-5}$ cm$^2$/sec; the intermediate curve (· · · · · ·) is generated using $D_{O_2, eff} = D_{O_2, SF} + D_{rbc}(1+M)$ where $M = 26$ and $\gamma_{avg} = 773$ sec$^{-1}$; the bottom curve (--- ---) is generated using $D_{O_2, eff} = D_{O_2, SF} + D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b) and $\gamma_{avg} = 773$ sec$^{-1}$. 

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Figure 4.25: Oxygen uptake by RBC suspensions with a $H_D$ of 30% and $P_{50}$ of 27 mmHg flowing at 12 µl/hr in a 27-µm-diameter membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sat}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation of experiments generated by simplified DM: the bottom curve (-----) is generated using $D_{O_2,eff}=2.25 \times 10^{-5}$ cm²/sec; the intermediate curve (- - - - -) is generated using $D_{O_2,eff}=D_{O_2,SF}+D_{rbc}(1+M)$ where $M=26$ and $\gamma_{avg}=773$ sec⁻¹; the upper curve (---) is generated using $D_{O_2,eff}=D_{O_2,SF}+D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b) and $\gamma_{avg}=773$ sec⁻¹.
**Figure 4.26:** Oxygen release from RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 27 mmHg flowing at 12 μl/hr in a 27-μm-diameter membrane tube at 37 °C. The left panel gives the space average $O_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean $O_2$ saturation ($S_{mm}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation of experiments generated by simplified DM: the upper curve (-----) is generated using $D_{O2,eff}=2.25 \times 10^{-5}$ cm$^2$/sec; the intermediate curve (- - - - -) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+M)$ where $M=18$ and $\gamma_{av}=773$ sec$^{-1}$; the bottom curve (---) is generated using $D_{O2,eff}=D_{O2,SF}+D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b) and $\gamma_{av}=773$ sec$^{-1}$.
**Figure 4.27:** Oxygen uptake by RBC suspensions with a $H_D$ of 20% and $P_{50}$ of 20 mmHg flowing at 23 µl/hr in a 27-µm-diameter membrane tube at 37 °C. The left panel gives the space average O$_2$ saturation ($S_{sa}$) at different axial positions along the artificial membrane tube; and the right panel, the mixed mean O$_2$ saturation ($S_{muv}$). Circles: experimental results, mean ± S.D. for 8 replicate experiments. Curves: theoretical simulation of the experiments generated by simplified DM: the bottom curve (-----) is generated using $D_{O2,eff}$=2.25x10$^{-5}$ cm$^2$/sec; the intermediate curve (- - - -) is generated using $D_{O2,eff}$=$D_{O2,5F}$+$D_{rbc}(1+M)$ where $M$=18 and $\gamma_{avg}$=1482 sec$^{-1}$; the upper curve (--- ---) is generated using $D_{O2,eff}$=$D_{O2,5F}$+$D_{rbc}(1+m)$ where $m$ is given by Equation (2.19b) and $\gamma_{avg}$=1482 sec$^{-1}$. 
Table 4.8

Comparison of Boland et al.'s oxygen transfer data to the simulation results generated by simplified DM using three different values of \( D_{O2,eff} \). Both the maximum deviation and algebraic deviation\(^*\) of the numerical calculations from the experimental data are given here for \( S_{sa} \), space average \( O_2 \) saturation.

<table>
<thead>
<tr>
<th>( D_{O2,eff} )</th>
<th>( D_{O2,pt}=D_{O2,SF} )</th>
<th>( D_{O2,pt}=D_{O2,SF}+D_{rbc}(1+M) )</th>
<th>( D_{O2,pt}=D_{O2,SF}+D_{rbc}(1+m) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error</td>
<td>lmax. deviation</td>
<td>algebraic deviation</td>
<td>lmax. deviation</td>
</tr>
<tr>
<td>Run R1</td>
<td>0.03</td>
<td>0.02 ↓*</td>
<td>0.16</td>
</tr>
<tr>
<td>Run U1</td>
<td>0.05</td>
<td>0.02 ↑</td>
<td>0.28</td>
</tr>
<tr>
<td>Run R2</td>
<td>0.05</td>
<td>0.02 ↑</td>
<td>0.12</td>
</tr>
<tr>
<td>Run U2</td>
<td>0.10</td>
<td>0.04 ↑</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\(^*\) The algebraic deviation is calculated as \( \sum_{i=1}^{N} (\text{deviation})/N \) where \( N \) is the number of data points.

\( \downarrow \) indicates that a model on the average under predicts the amount of transport, and \( \uparrow \) indicates that a model on the average over predicts the amount of transport.
and excursions from straight streamlines are highly suppressed in small vessels (Gaehgens et al., 1980). Therefore, the augmentation of oxygen transport from these mechanisms would tend to be less than that observed in the large vessels.

4.4 Summary

As mentioned previously, all the parameters in Nair et al.'s model are either physical properties or are determined by ways independent of the oxygen transport experiments. Hence, it is a predictive model that has been validated by well-defined experiments in microvessels of 27 µm and 100 µm diameters (Nair et al., 1989). Because the simplified DM presented here is in excellent agreement with Nair et al.'s model, it is concluded that the simple 1 PDE model presented here accurately predicts oxygen transport rates by flowing blood in microvessels. The simple model has the advantage of being more tractable mathematically and easier to apply in practice. These models have been verified for application to microvessels of 25-100 µm, and they may be assumed to apply to larger diameters for laminar flow. However, the models should be used with caution if applied to smaller microvessels where the flow and mass transfer characteristics are different than those in the larger vessels.

The predictions of all three models, CMAug, CMequ and simplified DM, prove to be in good agreement with the data obtained by Diller et al. (1980) and Schmukler and Chien (1985) for vessels with diameters of 100 µm and larger. Shear-induced augmentation is established to be important in vessels of 300 µm diameter and larger and under the conditions of operating both along the steepest portion of the ODC (m >150) and high shear rate (γavg≥1000 sec⁻¹). This transport enhancement is found to be insignificant in small microvessels (diameters of 30 µm and less). This result, however, raises the question: in what tube diameter range does the shear-induced augmentation
mechanism cease to be important? Schmukler and Chien’s result was obtained for low hematocrit suspensions ($H_D=1.5\%$ to $10\%$) where shear-induced dispersive migration of red cells due to cell collisions is not expected to play an important role (Zydeny and Colton, 1988). Therefore, this work does not exclude the possibility that shear-induced augmentation of oxygen transport is of significance for $100\mu m$-diameter vessels with higher hematocrits.

Another finding is that both continuum models underestimate the resistance to oxygen transport by flowing blood in small microvessels (diameter of $30 \mu m$ and less) and thus over predict the amount of transfer; while the discrete model, as previously stated, gives good agreement with the experimental measurements (space-averaged oxygen saturations). In these small vessels, the characteristic vessel dimension and the red cell size are of comparable size, and the cell-depleted layer constitutes a measurable fraction of the circular lumen. Thus, assumptions that hemoglobin is uniformly distributed over the tube and that the RBC velocity is described by a Poiseuille distribution are not valid. The discrete nature of blood begins to play more important role in determining the transport resistance for oxygen uptake and release by blood in this diameter regime. It is worth noting that, however, when the models are considered based on the mixed-mean or flow-average fractional saturation of hemoglobin (see the left panels of Figures 4.18 - 4.21), CMequ gives predictions that are reasonably close to those generated by simplified DM; while CMaug still seriously over predicts the amount of transport.
CHAPTER 5

THE ANION TRANSPORTER

Because the rate of chloride shift may indeed be rate-limiting under certain conditions in vivo, a basic understanding of the exchange mechanism is needed to learn about the effect of alterations in anion exchange kinetics on overall CO₂ transport by blood. Hence, the purpose of this chapter is to formulate a well-founded mathematical relationship to represent RBC anion exchange. In Section 5.1, the established characteristics on anion transporter are briefly described; and three approaches for quantitative characterization of Cl⁻/HCO₃⁻ exchange are considered: (1) a phenomenological permeability coefficient approach with appropriate chemical potential gradients, (2) a Michaelis-Menten type of approach and (3) a carrier-mediated type of model derived based on a “ping-pong” mechanism. Methods for measurements of anion exchange are briefly discussed in Section 5.2 to secure background information on the experimental techniques. In addition, a discussion on the experimental measurements carried out in both continuous flow (Klocke, 1976) and stopped-flow rapid-reaction devices (Weith, 1979; Illsley and Verkman, 1987; Lemon, 1989), and on development of mathematical models to describe these systems is included. Application of the anion flux expressions developed in Section 5.1 to analyze data is discussed in Section 5.3 with regard to the determination of kinetic parameters. The assessments of the anion exchange models are carried out by direct comparison of results from mathematical simulations with experimental data from the literature.
5.1 Mathematical Analysis of The Anion Transporter

5.1.1 Established Conditions on The Anion Transporter

There are reported to be 0.8-1.2×10^6 band 3 monomers per RBC - based on the number of stilbene binding sites per RBC depending on the individual donor. Stilbene competitively inhibits anion exchange when bound to the transporters (Gunn et al., 1973). Most of the experimental evidence on the exchange kinetics point toward the single-site “ping-pong” mechanism with obligatory exchange (Cabantchik et al., 1978; Lowe and Lambert, 1983; Falke and Chan, 1985; Jennings, 1985; Frohlich and Gunn, 1986; Passow, 1986; Jennings, 1989). The general features of the ping-pong mechanism are illustrated in Figure 5.1. In the ping-pong mechanism, the anions take turns crossing the membrane rather than switching place simultaneously; and the protein has two structurally distinct states, an inward-facing state and an outward-facing state. Thus it is an alternating site transporter possessing a single transport site which is alternatively exposed to the opposite sides of the membrane. This site can only cross the membrane when it is occupied by a substrate anion; it then undergoes a conformational change to face the opposite side of the membrane and releases the transported ion. The transported site can now bind another (or the same) anion and return to the original membrane face, release the anion to complete a cycle of anion exchange.

Although there are arguments for a sequential mechanism (two coupled transport sites, one on each side of the membrane that must be occupied for the simultaneous and reciprocal transfer of both anions) (Salhany and Rauenbuehler, 1983), the characteristics of the ping-pong mechanism has been experimentally demonstrated by various investigators. For instance, Jennings (1982) showed that a gradient of chloride concentration (with Cl− higher inside than outside of the cells) appeared to recruit anion carriers to an outward-facing conformation; and this behavior is that expected for a ping-
Figure 5.1: Diagram of a ping-pong mechanism for anion transport. Shown here is the exchange of an intracellular Cl\(^-\) for an extracellular HCO\(_3\)^-.

pong mechanism. If there are no transportable anions on one side of the membrane, all the transport sites will be recruited to this side by transporting an anion across to this side but are locked in this position and not able to transport one back because of the lack of substrate ion with which the transporter can form a complex. On the other hand, the sequential mechanism would require that half of the transport sites be on each side of the
membrane, and the fraction of the loaded sites would depend on the substrate concentrations on the two sides.

Other evidence came from the experimental demonstration of the half-turnover of the transporters, i.e. the transport of $10^6$ Cl$^-$ ions per RBC from inside to the outside when $10^6$ transport sites are recruited from the inside to the outside and blocked by an external irreversible inhibitor. Jennings (1982) demonstrated this by adding RBC ghosts (resealed erythrocytes) containing a low concentration of $^{36}$Cl$^-$ to a chloride and bicarbonate-free medium containing sulfate. There was a rapid loss of about $10^6$ Cl$^-$ per cell equivalent to one half-turnover of each transport site. Because SO$_4^{2-}$ was transported very slowly (Schnell et al., 1977), the ping-pong mechanism predicts an initial "burst" of Cl$^-$ due to one-half of a cycle followed by a much slower exchange of extracellular SO$_4^{2-}$ for intracellular Cl$^-$. Exchange of Cl$^-$ for SO$_4^{2-}$ was found to be accompanied by movement of protons with a stoichiometry of one proton plus one sulfate, HSO$_4^-$, to one chloride. This experimental evidence supports the notion that translocation of an anion from one side of the membrane to the other does not require simultaneous translocation of an anion in the opposite direction, i.e. the efflux half-cycle and influx half-cycle are separated, as specified in the ping-pong model. Moreover, because the number of anion transporters per RBC is $10^6$, this observation also suggested that the stoichiometry of anion transport is one ion per translocation per band 3 monomer.

Chloride binding to band 3 had also been studied using $^{35}$Cl$^-$ nuclear magnetic resonance (NMR) (Falke and Chan, 1985). Their results showed that there is a class of high-affinity Cl$^-$ binding sites that behave as the transport site might behave with respect to displacement by other anions, inhibitors and recruitment. However, the previously postulated modifier site, which was proposed by Dalmark (1976) to explain the inhibition of anion exchange at large anion concentration, was NMR-invisible and had
no effects on Cl\(^-\) binding to the transport site. Their results are quantitatively consistent with the ping-pong model, which states that the transport site is the only site involved in the transport cycle. Therefore, mechanisms with a ternary complex between band 3 and two chloride ions seem to be excluded by these observations.

5.1.2 Previous Approaches

5.1.2.a Constant Field Electrodiffusion Approach

Previously, the exchange of HCO\(_3\)^- and Cl\(^-\) had generally been regard as passive electrodiffusion. Various investigators (Crandall et al., 1971; Chow et al., 1976; Klocke, 1976; Salathe et al., 1981) recognized that RBC anion exchange process involves some form of membrane-anion interaction, and that passive diffusion down electrochemical gradient implies a specification of a mechanism. As a result, they used a phenomenological approach in which appropriate phenomenological permeability coefficients (\(P_{HCO3}\) and \(P_{Cl}\)) were introduced to quantitatively characterize HCO\(_3\)^-/Cl\(^-\) exchange under physiological conditions. Using Goldman's assumption of a linear decrease in electrical potential across the cell membrane, flux of a univalent ion across the RBC membrane is equal to (Goldman's equation which is given in Biophysical Chemistry of Membrane Functions by Kotyk et al., 1988)

\[
Flux_{HCO3} = \frac{P_{HCO3} F \Psi_m}{RT} \left[ \frac{[HCO3^-]_{pl} - [HCO3^-]_{rbc} \exp \left( - \Psi_m F/RT \right)}{1 - \exp \left( - \Psi_m F/RT \right)} \right]
\]  

(5.1)

where the intracellular and extracellular concentrations are expressed in (mole/liter RBC) and (mole/liter plasma), respectively; \(\Psi_m\) is the membrane potential; and \(P_{HCO3}\) is the permeability of HCO\(_3\)^- which was obtained based on experimental kinetic data. With appropriate concentrations and permeability, Equation (5.1) can also be written for Cl\(^-\)
flux. Upon imposing the electroneutrality condition, i.e. requiring the fluxes of \( \text{HCO}_3^- \) and \( \text{Cl}^- \) to sum to zero, the membrane potential can then be expressed as

\[
\Psi_m = -\frac{RT}{F} \ln \left( \frac{P_{\text{Cl}} [\text{Cl}^-]_{pl} + P_{\text{HCO}_3} [\text{HCO}_3^-]_{pl}}{P_{\text{Cl}} [\text{Cl}^-]_{rbc} + P_{\text{HCO}_3} [\text{HCO}_3^-]_{rbc}} \right)
\]  
\hspace{1cm} (5.2)

Other implicit assumptions involved in deriving Equation (5.2) are that activity coefficients are identical both inside and outside the RBCs, and that permeabilities are independent of the direction of ionic movement.

Computation of the permeabilities using the constant field assumption can be simplified by conducting the initial rate approach (Chow et al., 1976). Assuming that \( P_{\text{HCO}_3} \) and \( P_{\text{Cl}} \) remain constant at any given \( pH \) in spite of different experimental conditions, the initial membrane potential depends upon the intra- and extracellular concentrations present immediately after mixing of reactants. For example, at the start of experiments with \( \text{Cl}^- \)-loaded cells, since \([\text{Cl}^-]_{pl}\) and \([\text{HCO}_3^-]_{rbc}\) are small, if both permeabilities are not widely different Equation (5.2) simplifies to

\[
\Psi_m = -\frac{RT}{F} \ln \left( \frac{P_{\text{HCO}_3} [\text{HCO}_3^-]_{pl}}{P_{\text{Cl}} [\text{Cl}^-]_{rbc}} \right)
\]  
\hspace{1cm} (5.3)

and the membrane potential is dependent on \([\text{Cl}^-]_{rbc}\) and \([\text{HCO}_3^-]_{pl}\) and the respective permeabilities. In addition, the total flux of \( \text{HCO}_3^- \) from plasma to RBC was calculated from the initial kinetic measurements. Hence, by varying the initial reactant compositions, at least two sets of independent data were required here, the three unknowns \((P_{\text{HCO}_3}, P_{\text{Cl}} \text{ and } \Psi_m)\) can then be calculated. Klocke (1976), on the other hand, used the integral method to analyze his kinetic data. Using Equations (5.1) and (5.2) and assumed permeabilities forward integration was conducted from the initial
conditions to give a relation between composition of the reaction mixture and time. A trial-and-error procedure was continued until the best fit of the theoretical curves to the observed data was achieved by the method of least square. Table 5.1 summarizes the permeability coefficients calculated by Chow et al. (1976) and Klocke (1976):

**Table 5.1:** Permeabilities of HCO$_3^-$ and Cl$^-$ for anion exchanges in RBCs calculated based Goldman’s solution for the electrodiffusion of ions.

<table>
<thead>
<tr>
<th>Experimenters</th>
<th>$P_{HCO3}$ (cm/sec)</th>
<th>$P_{Cl}$ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 °C</td>
<td>25 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>Chow et al.</td>
<td>3.8x10$^{-6}$</td>
<td>1.2x10$^{-4}$</td>
</tr>
<tr>
<td>Klocke</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

It is interesting to note that $P_{Cl}$ obtained by Klocke (1976) averaged 1.1x10$^{-4}$ cm/sec and were not significantly correlated with experimental conditions; in contrast, to the relatively constant value of $P_{Cl}$, the $P_{HCO3}$ varied widely. In addition the relatively low temperature dependence of $P_{Cl}$ is difficult to reconcile with the values of the activation energy that were reported in the literature.

However, discrepancies of several orders of magnitude between the Cl$^-$ permeability as measured by isotope exchange and by net (or conductive) flow with direct electrical measurements practically ruled out the possibility that anions as such diffuse across the membrane (Cabantchik et al., 1978). Those data alone with other experimental evidence suggest that the exchange mechanism is carrier-mediated; the carrier is capable of one-for one exchange of anions, but does not permit the net flow of anions across the membrane. As a result, these permeability values are only “effective” and must be interpreted accordingly. Therefore, modifications needed to be introduced
to include the possibility of passive mediated-transport system. Such transport system
includes a specific protein which contains binding site(s) complementary to the
substrate(s) transported and serves as a carrier of the substrate(s) (Lehninger, 1975). In
addition, the process is completely reversible, and the movement of the substrate(s) may
be in either direction, depending on the relative concentration(s) of the substrate(s) in the
two compartments.

5.1.2.b  Michaelis-Menten Kinetic Approach

Because saturation effects were observed in the HCO₃⁻/Cl⁻ exchange system,
some investigators (Lambert and Lowe, 1978 and 1980; London et al., 1987) used
Michaelis-Menten equation to characterize their data. Basically the Michaelis-Menten
equation expresses the mathematical relationship between the initial rate ($v_o$) of an
enzyme-catalyzed reaction, the concentration of the substrate ([S]₀), and certain
characteristics of the enzyme. This equation is derived by making the steady-state
approximation for the intermediate enzyme-substrate complex and utilizing the
conservation equation for total enzyme concentration.

$$v_o = \frac{v_{\text{max}} [S]_o}{K_M + [S]_o}$$  \hspace{1cm} (5.4)

where $v_{\text{max}}$ is the maximum reaction rate and $K_M$ is known as the Michaelis constant.
An important numerical relationship emerges from the above equation in the special case
when $v_o=0.5v_{\text{max}}$; consequently, $K_M$ is equal to the concentration of $[S]_o$ which gives
the half maximum reaction rate. $v_{\text{max}}$ and $K_M$ can be determined by a reciprocal plot
which is based on the rearrangement of Equation (5.4) into the following form.

$$\frac{1}{v_o} = \frac{1}{v_{\text{max}}} + \frac{K_M}{v_{\text{max}} [S]_o}$$  \hspace{1cm} (5.5)
If the data fit the model, a plot of $1/v_o$ versus $1/[S]_o$ should be linear with a slope of $K_M/v_{max}$ and intercept of $1/v_{max}$. Although Michaelis-Menten expression is applicable to a wide variety of enzyme catalyzed reactions; it is not appropriate for reversible reactions and multiple-substrate reactions. As a result, the values for $K_M$ and $v_{max}$ reported in the literature can only be considered as effective and apparent because they were obtained by assuming that this saturable carrier system is analogous to an enzyme which obeys Michaelis-Menten kinetic.

Lowe and Lambert (1983) analyzed various studies (Brahm, 1977; Lambert and Lowe, 1980; Weith and Brahms, 1980) and reported the apparent values for $K_M$ and $v_{max}$ which were extracted from those experimental studies (see Table 5.2). Lowe and Lambert (1983) had also attempted to make some sort of correction on the fact that they were using a single-substrate kinetic expression to describe an bisubstrate reaction. For instance, in a case where the driving force was set up for the net exchange of intracellular $Cl^-$ with extracellular $HCO_3^-$, they regarded competition between $Cl^-$ and $HCO_3^-$ at extracellular sites as lowering the effective concentration of $HCO_3^-$ by a factor of $1/(1+[Cl^-]_p/K_{I,Cl})$ where $K_{I,Cl}$ is the dissociation constant of the carrier-$Cl^-$ complex. In an analogous fashion, intracellular $HCO_3^-$ compete with $Cl^-$ for transport sites; and this is treated as having effect of reducing the rates of exchange by a factor of $1/(1+[HCO_3^-]_{rcd}/K_{I,HCO3})$ where $K_{I,HCO3}$ is the dissociation constant of the carrier-$HCO_3^-$ complex. Another correction applied in this case for obtaining the extrapolated $v_{max}$ was to assume Michaelis-Menten kinetics with the apparent $K_M$ and extrapolate $v_{max}$ to infinite intracellular $Cl^-$ concentration. The apparent discrepancies in the results of different workers probably arise, in part at least, from the different conditions of $pH$ and competitive effects between anions prevailing under the different experimental conditions. More importantly, the issue is that Michaelis-Menten equation is not
applicable for characterizing the anion transporter because of the fundamental assumptions and specific mechanism that are associated with this kinetic model.

**Table 5.2:** Rates (observed \( v_{\text{max}} \) and extrapolated \( v_{\text{max}} \)) and concentration of \( \text{Cl}^- \) and \( \text{HCO}_3^- \) giving half maximum rates (\( K_M \)) of anion exchanges in human red blood cells or red cell ghosts at 37 °C.

<table>
<thead>
<tr>
<th>Anion Exchange</th>
<th>Apparent ( K_M )</th>
<th>Observed ( v_{\text{max}} )</th>
<th>Extrapolated ( v_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(^-)/Cl(^-) exchange(\dagger)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brahman</td>
<td>65.0</td>
<td>56.6</td>
<td>81.2</td>
</tr>
<tr>
<td>Weith and Brahman</td>
<td>---</td>
<td>49.0</td>
<td>68.3</td>
</tr>
<tr>
<td>HCO(_3)(^-)/HCO(_3)(^+)(\dagger)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weith and Brahman</td>
<td>43.0</td>
<td>24.0</td>
<td>30.3</td>
</tr>
<tr>
<td>Cl(^-)/HCO(_3)(^+)(\dagger)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weith and Brahman</td>
<td>---</td>
<td>26.0</td>
<td>32.8</td>
</tr>
<tr>
<td>Lambert and Lowe</td>
<td>11.1</td>
<td>10.2</td>
<td>61.6</td>
</tr>
<tr>
<td>HCO(_3)(^-)/Cl(^+)(\dagger)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weith and Brahman</td>
<td>---</td>
<td>48.0</td>
<td>66.9</td>
</tr>
</tbody>
</table>

\(\dagger\) For hetero-exchange the first named anion is intracellular.

\(^*\) Uncorrected for the inhibitory effects of anions. \( K_M \) is for HCO\(_3\)\(^-\) in the case of heteroexchange.

\(^\dagger\) With anions usually present at 150-165 mM inside and outside the cell.

\(^\#\) Extrapolated assuming Michaelis-Menten kinetics and the apparent \( K_M \) stated.

### 5.1.3 Simplified Ping-pong Mechanism

The scheme illustrated in Figure 5.2 was proposed by Frohlich and Gunn (1986). They attempted to use the minimal number of reaction steps and different kinetic states of the anion transporter that are necessary to describe the one-for-one
exchange of the anions. They decomposed the exchange process into three steps: (1) binding of the anion to the transport molecule at one surface of the membrane, (2) translocation of the anion across the membrane, and (3) dissociation of the anion from the transport molecule at the opposite surface. Reaction rate expression for enzymatic reactions are usually derived by making the steady-state approximation for the intermediate enzyme-substrate complexes. This is an appropriate assumption when the substrate concentration greatly exceeds that of the enzyme (the usual laboratory situation) or when there is both a continuous supply of reactant and a continuous removal of products (the usual cellular concentration).

**Figure 5.2:** Description of the catalytic cycle of anion exchange according to the ping-pong scheme.

![Diagram](image)

where

$T_{rbc}$ and $T_{pl}$ are the unloaded forms of the transporter in which a single anion binding/transport site is accessible to only intra- or extracellular anions, respectively,

$TX_{rbc}$, $TX_{pl}$, $TY_{rbc}$ and $TY_{pl}$ denote the complexes of the transporter with the anions $X$ ($Cl^-$) and $Y$ ($HCO_3^-$).
$k_1$, $k_3$, $k_4$ and $k_6$ are the association rate constants.

$k_{-1}$, $k_{-3}$, $k_{-4}$ and $k_{-6}$ are the dissociation rate constants.

$k_2$ and $k_5$ are the outward translocation rate constants.

$k_{-2}$ and $k_{-5}$ are the inward translocation rate constants.

Based on the above kinetic scheme, Frohlich and Gunn (1986) derived the differential equations which describe how the concentrations of the different transporter conformations change with the substrate and product concentrations. Then they applied the generalized steady-state analysis (i.e., the concentrations of different states of the transporter are constant) to solve for the steady-state concentrations of different states of the anions. The resulting concentrations are given in Frohlich and Gunn (1986). $T_{rbc}/T_{tot}$, $T_{pl}/T_{tot}$, $TX_{rbc}/T_{tot}$, $TX_{pl}/T_{tot}$, $TY_{rbc}/T_{tot}$ and $TY_{pl}/T_{tot}$ are functions of $\{k_i\}$, where $i=\pm 1, \ldots, \pm 6$; and $X_{rbc}, X_{pl}, Y_{rbc}, Y_{pl}$ and $T_{tot}$ is the total number of band 3 transporter molecules; these expressions give the fraction of the transporter molecules that are in the given state (or the probability of a given transporter molecule being in a particular state). The flux equation which describes the anion concentrations on the two sides of the membrane can be constructed from the steady concentrations by expressing the net reaction rate between any two adjacent points in the reaction scheme (Figure 5.2).

$$v = \frac{\text{Flux}_{HCO_3}}{T_{tot}} = k_1 \frac{T_{rbc}}{T_{tot}} \frac{X_{rbc}}{T_{tot}} - k_{-1} \frac{TX_{rbc}}{T_{tot}} = k_2 \frac{TX_{rbc}}{T_{tot}} - k_{-2} \frac{TX_{pl}}{T_{tot}} = \text{etc.}$$

(5.6)

where

$v$ is the turnover rate.

$\text{Flux}_{HCO_3}$ is the net flux of extracellular $\text{HCO}_3^{-}$ entering the RBCs or equivalently, the net flux of intracellular $\text{Cl}^{-}$ entering the plasma.

$T_{tot}$ is the total number of band 3 transporter molecules per RBC (0.8-1.2x10$^6$).
\[ v = (X_{rbc} Y_{pl}) \left[ c X_{rbc} + \frac{(bc)}{a} X_{pl} + \frac{(bc)}{d} Y_{rbc} + b Y_{pl} + c K_3 \left(1 + \frac{1}{K_2} \right) X_{rbc} Y_{pl} \right. \]

\[ + b K_6 \left(1 + \frac{1}{K_5} \right) Y_{rbc} Y_{pl} + (g + f) X_{rbc} Y_{pl} + \frac{(K_3 K_5 K_6)}{K_1 K_2 K_4} (h + e) X_{pl} Y_{rbc} \right]^{-1} \]

\[ - (X_{pl} Y_{rbc}) \left[ d X_{pl} + \frac{a d}{b} X_{rbc} + \frac{a d}{c} Y_{pl} + a Y_{rbc} + d K_1 (1 + K_2) X_{rbc} X_{pl} \right. \]

\[ + a K_4 (1 + K_5) Y_{rbc} Y_{pl} + (h + e) X_{pl} Y_{rbc} + \frac{(K_1 K_2 K_4)}{K_3 K_5 K_6} (g + f) X_{rbc} Y_{pl} \right]^{-1} \]

(5.7)

where

\[ X_{rbc} = [Cl^-]_{rbc}, Y_{rbc} = [HCO_3^-]_{rbc}, X_{pl} = [Cl^-]_{pl} \text{ and } Y_{pl} = [HCO_3^-]_{pl}. \]

\[ a = \frac{k_{-1} k_{-2} + k_{-1} k_{-3} + k_{-2} k_{-3}}{k_{-1} k_{-2}}, e = \frac{k_{-1} + k_{-2} + k_{-3}}{k_{-1} k_{-2}} \]  

(5.8a)

\[ b = \frac{k_{-1} k_{-2} + k_{-1} k_{-3} + k_{-2} k_{-3}}{k_{1} k_{2} k_{3}}, f = \frac{k_{2} + k_{-2} + k_{-3}}{k_{2} k_{3}} \]  

(5.8b)

\[ c = \frac{k_{-4} k_{5} + k_{-4} k_{6} + k_{-5} k_{-6}}{k_{4} k_{5} k_{6}}, g = \frac{k_{5} + k_{-5} + k_{-6}}{k_{5} k_{6}} \]  

(5.8c)

\[ d = \frac{k_{-4} k_{5} + k_{-4} k_{6} + k_{-5} k_{-6}}{k_{4} k_{5} k_{6}}, h = \frac{k_{-4} + k_{5} + k_{-5}}{k_{4} k_{5}} \]  

(5.8d)

\[ K_j = \frac{k_j}{k_{-j}} \quad (j = 1, 2, ..., 6) \]

Equation (5.7) is recited here for the purpose of a discussion on how additional assumptions are introduced to simplify the flux expression. Detailed description of the exchange kinetic at this level would be too cumbersome; as seen from Equation (5.7), it is rather unyielding. The nature of the transmembrane processes could depend largely on the identity of the reaction or reactions that are rate-limiting in the transport cycle. Falke and coworkers (1985a, 1985b) utilized \(^{35}\text{Cl}^-\) nuclear magnetic resonance (NMR) and \(^{37}\text{Cl}^-\) NMR to set lower limits on the rates of chloride binding and dissociation at
the saturated inward- and outward-facing band 3 transport sites. The physical basis of the NMR technique is the large difference in the spectral widths of the bound and free Cl\(^-\), the spectral width of Cl\(^-\) bound to a macromolecule is typically \(\geq 10^4\) times than the spectral width of solution Cl\(^-\). As a result when solution Cl\(^-\) visits a macromolecular binding site sufficiently rapidly, the solution Cl\(^-\) NMR resonance can be measurably broadened. When line broadening is observed, it contains information on the rate of Cl\(^-\) migration between the binding site and the bulk solution. The exchange of Cl\(^-\) between a binding site and solution can be slow, intermediate, or rapid on the Cl\(^-\) NMR time scale. For slow exchange the \(^{35}\)Cl\(^-\) or \(^{37}\)Cl\(^-\) line broadening exactly specifies the exchange rate. At both 0-3 and 37 °C, their NMR data specified that Cl\(^-\) binding and dissociation at the saturated sites are not rate-limiting, indicating that translocation of bound Cl\(^-\) across the membrane is the slowest step in the overall transport cycle. The Cl\(^-\) binding and dissociation rates determined by Falke \textit{et al.} (1985) allow important features of the kinetic equation for a ping-pong transport cycle to be described (see Figure 5.3). However, it should be mentioned that at the inward-facing transport site the turnover rate at 37 °C has become comparable to the rate of Cl\(^-\) binding and dissociation, so these rates need to be examined more closely.

If the translocation step is also relatively slower than the HCO\(_3^-\) dissociation step, one can then simplify the kinetic analysis by letting the translocation step be rate limiting or alternatively by letting the association and dissociation reactions be at equilibrium and by eliminating terms in the kinetic equation which are now known to be negligibly small. The simplified version of lumped kinetic parameters, \(a \cdot h\), is as followings:

\[
a = \frac{1}{k_{-2}k_3} \quad e = \frac{1}{k_{-2}} \quad \text{(5.9a)}
\]
Figure 5.3: Transport cycle of the anion transporter. Falke et al.'s $^{35}$Cl$^{-}$ and $^{37}$Cl$^{-}$ NMR data specifies that at both low and physiological temperatures, the binding and dissociation events at both orientations of the transport site are measurably faster than the translocation step.
\[ b = \frac{1}{k_2 k_1} \quad f = \frac{1}{k_2} \quad (5.9b) \]
\[ c = \frac{1}{k_5 k_4} \quad g = \frac{1}{k_5} \quad (5.9c) \]
\[ d = \frac{1}{k_5 k_6} \quad h = \frac{1}{k_5} \quad (5.9d) \]

In addition, the principle of microscopic reversibility for a passive system requires that for the hexagonal scheme of Figure 5.2: \( k_1 k_2 k_3 k_4 k_5 k_6 = k_{-1} k_{-2} k_3 k_4 k_5 k_6 \) which in turn becomes: \( K_1 K_2 K_4 / K_3 K_5 K_6 = 1 \). Upon introducing above assumptions, the flux equation (Equation (5.7)) is simplified to the following:

\[
\nu = (X_{rbc} Y_{pl}) \left[ \frac{X_{rbc}}{k_5 K_4} + \frac{K_3 X_{pl}}{k_5 K_1 K_2 K_4} + \frac{K_5 K_6 Y_{rbc}}{k_2 K_1 K_4} + \frac{Y_{pl}}{k_2 K_1} + \frac{K_3}{k_5 K_4} \left( \frac{1}{K_2} \right) X_{rbc} X_{pl} \right]^{-1}
\]

\[
+ \frac{k_6}{k_2 K_1} \left( \frac{1}{k_5} + \frac{1}{k_2} \right) Y_{rbc} Y_{pl} + \left( \frac{1}{k_5} + \frac{1}{k_2} \right) X_{rbc} Y_{pl} + \left( \frac{1}{k_5} + \frac{1}{k_2} \right) Y_{pl} Y_{rbc} \right]^{-1}
\]

\[
- (X_{pl} Y_{rbc}) \left[ \frac{X_{pl}}{k_5 K_6} + \frac{K_4 Y_{pl}}{k_5 K_3 K_5 K_6} + \frac{K_1 K_2 X_{rbc}}{k_5 K_3 K_6} + \frac{Y_{pl}}{k_2 K_3} + \frac{K_1 (1 + K_2)}{k_5 K_6} X_{rbc} X_{pl} \right]
\]

\[
+ \frac{k_4 (1 + K_5)}{k_2 K_3} \left( \frac{1}{k_5} + \frac{1}{k_2} \right) Y_{rbc} Y_{pl} + \left( \frac{1}{k_5} + \frac{1}{k_2} \right) X_{rbc} Y_{pl} + \left( \frac{1}{k_5} + \frac{1}{k_2} \right) Y_{pl} Y_{rbc} \right]^{-1}
\]

where

- \( X_{rbc} \) denotes \([Cl^-]_{rbc}\), \( Y_{rbc} \) denotes \([HCO_3^-]_{rbc}\), \( X_{pl} \) denotes \([Cl^-]_{pl}\) and \( Y_{pl} \) denotes \([HCO_3^-]_{pl}\).
- \( K_j = k_j / k_{-j} \) for \( j = 1, 3, 4 \) and \( 6 \). \( K_1 \) and \( K_3 \) are the microscopic equilibrium association constants for Cl⁻-protein complex in the inward and outward membrane compartments, respectively. \( K_4 \) and \( K_6 \) are the microscopic equilibrium association constants for HCO₃⁻-protein complex in the outward and inward membrane compartments, respectively.
$k_2, k_{-2}(k_5, k_{-5})$, the rates of translocation of bound Cl$^-$ (HCO$_3^-$), are defined in the ping-pong model as the microscopic rate constant for conversion of an intracellular carrier-anion binary complex to an extracellular carrier complex and vice-versa.

It should be mentioned that inhibitory effects on anion exchange are not taken into account. Inhibitory effects can be divided into the following three types: (a) self inhibition by the high concentrations of the transported anions (Dalmark, 1976; Lambert and Lowe, 1980, Weith et al., 1980; Weith and Bjerrum, 1982), (b) competitive inhibition by other anions where the inhibitor competes for the same unloaded anion transporter (Dalmark, 1976; Lambert and Lowe, 1978), and (c) other inhibitors which inactive the anion carrier through predominantly non-competitive mechanism where the inhibitors bind to both the loaded and unloaded transporters (Lambert and Lowe, 1978 and 1980; Weith et al., 1980). The phenomena of self-inhibition has been observed at relatively high extracellular concentrations of Cl$^-$ and HCO$_3^-$ compared to the physiological concentrations. Therefore, it is reasonable for a first approximation to neglect it. The mechanism behind these different types of inhibition are still not known well. There are some apparent discrepancies in the results of different workers on the studies of competitive inhibition. This probably arises, in part at least, from the different conditions of pH, temperature and competitive effects between anions prevailing under different experimental conditions.

For a first order approximation of the anion exchange, additional assumptions are introduced to further simplify the expression for the flux (Equation (5.10)):

1. The translocation step is assumed to be the rate limiting step.
2. $k_{\text{trans}}$, the translocation rate constant, is assumed to be independent of the type of bound anions as well as independent of the direction of the translocation. Therefore, $k_{\text{trans}} = k_2 = k_{-2} = k_5 = k_{-5}$. 
(3) Equilibrium association constants ($K_A$s) for Cl\textsuperscript{-} and HCO\textsubscript{3}\textsuperscript{-} are assumed to be the same in both membrane compartments (i.e., facing-inward and facing-outward). Therefore, $K_A = K_1 = K_3 = K_4 = K_6$ (Lemon, 1989).

(4) In the literature, it has been reported that HCO\textsubscript{3}\textsuperscript{-}/Cl\textsuperscript{-} exchange is essentially independent of internal $pH$ in the range of intracellular $pH$ from 7.0 to 8.0. In addition, it is approximately independent of extracellular $pH$ in the range from 7.0 to 9.0 (Obaid and Crandall, 1979; Weith et al., 1980; Weith and Bjerrum, 1982; Weith et al., 1982). Thus, $k_{trans}$ and $K_A$ are assumed to be independent of $pH$.

Upon introducing assumptions (1) - (4), Equation (5.10) is simplified to the following equation which is mathematically more tractable and contains only two kinetic parameters, $k_{trans}$ and $K_A$.

$$Flux_{HCO_3} = T_{tot} k_{trans} K_A \left( [Cl^-]_{rbc} [HCO_3^-]_{pl} - [Cl^-]_{pl} [HCO_3^-]_{rbc} \right) \bigg/ \left\{ [Cl^-]_{rbc} + [Cl^-]_{pl} + [HCO_3^-]_{rbc} + [HCO_3^-]_{pl} + 2 K_A \left( [Cl^-]_{rbc} [Cl^-]_{pl} + [HCO_3^-]_{rbc} [HCO_3^-]_{pl} + [Cl^-]_{pl} [HCO_3^-]_{rbc} \right) \right\}$$

(5.11)

Equation (5.11) predicts that there is a net influx of HCO\textsubscript{3}\textsuperscript{-} into RBCs or a net efflux of Cl\textsuperscript{-} entering the extracellular medium as long as

$$\frac{[HCO_3^-]_{pl}}{[HCO_3^-]_{rbc}} > \frac{[Cl^-]_{pl}}{[Cl^-]_{rbc}}$$

(5.12a)

For a long time it has been known that the ratios of the inside and outside concentrations of the various permeable anions (and of the cation, H\textsuperscript{+}, because $[H^+]_{rbc} [OH^-]_{rbc} = [H^+]_{pl} [OH^-]_{pl}$) were approximately given by the Gibbs-Donnan relationship (Van Slyke et al., 1923).
\[ R_{GD} = \frac{[Cl^-]_{rbc}}{[Cl^-]_{pl}} = \frac{[HCO_3^-]_{rbc}}{[HCO_3^-]_{pl}} = \frac{[SO_4^{2-}]_{rbc}}{[SO_4^{2-}]_{pl}} = \frac{[OH^-]_{rbc}}{[OH^-]_{pl}} = \frac{[H^+]_{pl}}{[H^+]_{rbc}} \] (5.12b)

where \( R_{GD} \) is the so called "Donnan ratio". That is the ions behave as if they are all in equilibrium with the same membrane potential. This is exactly what would be predicted if the ions were to diffuse across the membrane in response to their electrochemical potential difference. Cabanchik et al. (1978) pointed out that for thermodynamic reasons even in the carrier model in which no electrical driving force is considered and in which ions are assumed to carry no current across the membrane, one would predict the same relationship between the equilibrium distribution ratio of the various ions. Therefore Equation (5.12a) derived from Equation (5.11) is consistent with the Donnan-type distribution of ions (Equation (5.12b)) which is approached at equilibrium when the net flux must be zero.

5.2 Experimental Validation of Flux Expression

5.2.1 Methods of Measurement of Cl⁻/HCO₃⁻ Exchange

The transmembrane movements of the Cl⁻ can be monitored without serious complications, but movements of HCO₃⁻ involve an additional feature which must be taken into account in any technique adopted. This complication is the involvement in the following equilibria: \( CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \). Basically, two methods of overcoming this difficulty during measurements of HCO₃⁻ movements had been used. Details of these techniques are discussed below, because in assessing the significance of results obtained using one of these methods in relation to those obtained using the other it is necessary to consider carefully the experimental procedures used.
5.2.1.a  *pH Changes and Jacobs-Stewart Cycle*

When an intracellular $\text{Cl}^-$ exchanges for an extracellular $\text{HCO}_3^-$, subsequent operation of Jacobs-Stewart Cycle (Figure 5.4) leads to production of one extracellular $\text{H}^+$ and the absorption of one intracellular $\text{H}^+$. In principle, it is therefore possible to measure $\text{Cl}^-/\text{HCO}_3^-$ exchange by following changes in extracellular $pH$, provided that the anion exchange is the rate-limiting step in the cycle and that $pH$ measuring technique has a sufficiently fast response. In human red blood cells under normal circumstances steps 2 and 3, as illustrated in Figure 5.4, are very much faster than anion exchange.

**Figure 5.4:** Operation of the Jacobs-Stewart cycle and rapid acidification of the extracellular medium by $\text{Cl}^-/\text{HCO}_3^-$ exchange after addition of packed RBCs to a $\text{Cl}^-$-free media which contains carbonic anhydrase enzyme.
(step 1) because intracellular hydration of CO₂ is catalyzed by carbonic anhydrase which is present at high activity levels in RBCs and the physical diffusion of CO₂ cross the membrane is very rapid. However, the rate of hydration of extracellular H₂CO₃ is much slower in comparison to the anion exchange process. This difficulty can be overcome by adding carbonic anhydrase to the extracellular medium in a concentration sufficient to leave Cl⁻/HCO₃⁻ exchange as the rate-limiting step in the cycle. The rate of change in \( pH \) which reflects the operation of Jacobs-Stewart cycle, can then be taken as a measure of the rate of Cl⁻/HCO₃⁻ exchange. The types of apparatuses and detection methods that were used by Chow et al. (1976), Obaid and Crandall (1979) and Lemon (1989) who measured the anion exchange with the method described above are summarized in Table 5.3.

5.2.1.b Uncatalyzed Hydration/Dehydration of CO₂

An alternative approach to measurement of the anion exchange is one in which the Jacobs-Stewart cycle is prevented by inhibiting the catalyzed hydration of CO₂ by carbonic anhydrase (Figure 5.5). When this is done, rates of CO₂ transport become very slow compared with rates of anion exchange and isotopically labeled HCO₃⁻ can be used to monitor HCO₃⁻ movement. The carbonic anhydrase activity was effectively eliminated by, firstly, using resealed red cell ghost from which most of the enzyme had been lost during lysis and, secondly, incorporating into the ghost an inhibitor of carbonic anhydrase (e.g., acetazolamide or 2,6-pyridine dicarboxylic acid which chelates the zinc ions). If the formation of intracellular \(^{14}\)CO₂ is not eliminated, the tracer will permeate the membrane in the form \(^{14}\)CO₂. Due to the high membrane permeability to CO₂, intra- and extracellular CO₂ will equilibrate rapidly, and this will lead to rapid loss of the tracer. Therefore, under the conditions described previously, after mixing of Cl⁻-containing cells to a Cl⁻-free medium that contains H\(^{14}\)CO₃⁻ ion, the influx of \(^{14}\)CO₂ would be very much slower than the influx of [\(^{14}\)C]bicarbonate and so
the rate of entry of radioactivity would be a good measure of \( \text{HCO}_3^- \) transport (Weith, 1979). Along this same line of conducting experiments in the presence of carbonic anhydrase inhibitor, \( \text{Cl}^- \)-sensitive fluorescent probe have also been used to follow the exchange (Illesley and Verkman, 1987). The types of apparatus and detection methods that were used by Klocke (1976), Weith (1979), Illesley and Verkman (1987) who measured the anion exchange with the method described above are synopsized in Table 5.3.

\[\begin{align*}
\text{H}^+ & \rightarrow \text{HCO}_3^- \\
\text{HCO}_3^- & \rightarrow \text{CO}_2 \\
\text{AT} & \quad \text{carbonic anhydrase inhibitor} \\
\text{Cl}^- & \rightarrow \text{Cl}^- \\
\text{Cl}^- & \rightarrow \text{HCO}_3^- \\
\text{H}^+ & \rightarrow \text{H}_2\text{O} \\
\text{H}_2\text{O} & \rightarrow \text{CO}_2 \\
\text{CO}_2 & \rightarrow \text{H}_2\text{O}
\end{align*}\]

**Figure 5.5:** Operation of the \( \text{Cl}^-/\text{HCO}_3^- \) exchange after intracellular carbonic anhydrase activity is blocked so that simultaneous movement of \( \text{CO}_2 \) is eliminated.
Table 5.3: Apparatuses, detection methods and experimental conditions in Cl⁻/HCO₃⁻ exchange experiments used by various investigators.

<table>
<thead>
<tr>
<th>Workers</th>
<th>Apparatuses</th>
<th>Detection methods</th>
<th>RBCs/ghosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow <em>et al.</em> (1976)</td>
<td>stopped-flow</td>
<td>pH electrode</td>
<td>intact RBCs</td>
</tr>
<tr>
<td></td>
<td>rapid-reaction</td>
<td></td>
<td>(Ht=16%)</td>
</tr>
<tr>
<td></td>
<td>device (T=5-40 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obaid and Crandall (1979)</td>
<td>rapid-reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>device (T=25 °C)</td>
<td>pyranine⁺ or SPQ⁺</td>
<td>intact RBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pH-sensitive or Cl⁻-sensitive fluorescent dye)</td>
<td>(Ht=6.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Workers</th>
<th>Apparatuses</th>
<th>Detection methods</th>
<th>RBCs/ghosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klocke (1976)</td>
<td>continuous-flow</td>
<td>chloridometer</td>
<td>intact RBCs</td>
</tr>
<tr>
<td></td>
<td>rapid-reaction</td>
<td>gas chromatography</td>
<td>(Ht=19%)</td>
</tr>
<tr>
<td></td>
<td>device (T=37 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weith (1979)</td>
<td>stopped-flow</td>
<td>tracer method</td>
<td>resealed ghosts</td>
</tr>
<tr>
<td></td>
<td>rapid-reaction</td>
<td>[¹⁴C]HCO₃⁻</td>
<td>(Ht=1%)</td>
</tr>
<tr>
<td></td>
<td>device (T=25 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illsley and Verkman (1987)</td>
<td>stopped-flow</td>
<td>SPQ</td>
<td>resealed ghosts</td>
</tr>
<tr>
<td></td>
<td>rapid-reaction</td>
<td>(Cl⁻-sensitive fluorescent probe)</td>
<td>(Ht=0.03%)</td>
</tr>
<tr>
<td></td>
<td>device (T=37 °C)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁺ Pyranine is 8-hydroxy-1,3,6-pyrene trisulfonic acid.
* SPQ denotes 6-methoxy-N-(3-sulfopropyl) quinolinium.
5.2.2 Description of Experimental Systems and Development of Analysis

5.2.2.a Klocke's Experimental System (1976)

Klocke studied Cl⁻/HCO₃⁻ exchange in a continuous-flow rapid-reaction apparatus at 37 °C. The Reynolds number was greater than 5000 so that turbulent flow was ensured to promote mixing. The residence time can be estimated from the flow rate and the distance travelled in the tube. Thus, one can model Klocke's system as an ideal, isothermal continuous flow stirred tank reactor. It is a two-compartment system between which there exists a transmembrane gradient for both anions, and this gradient is produced by having RBCs which were loaded with one ion reacted with an isoosmotic solution which contained the other ion. Carbonic anhydrase activity was inhibited, so that simultaneous movement of CO₂ can be avoided and free CO₂ concentration, [CO₂]ₚₙₙ, remained essentially constant during the experimental period. During sample collection, RBCs were trapped in the mesh of the filter, so that extracellular Cl⁻ and HCO₃⁻ concentrations can be determined. Extracellular Cl⁻ concentration was determined coulometrically. Total extracellular CO₂ content, [CO₂]ₚₙₙ, was measured with gas chromatography; then extracellular HCO₃⁻ concentration was calculated through the following relationship: [HCO₃⁻]ₚₙₙ = [CO₂]ₚₙₙ - [CO₂]ₚₙₙ, free.

The mathematical description of Klocke’s system follows:

**Intracellular phase**

\[
\left( [HCO₃⁻]_{rbc, ou} - [HCO₃⁻]_{rbc, in} \right) = \left( \frac{\delta}{V} \right)_{rbc} \text{Flux}_{HCO₃} \left( \frac{V_R}{Q} \right)
\]  

(5.13)
\[
([\text{Cl}^-]_{\text{rbc, out}} - [\text{Cl}^-]_{\text{rbc, in}}) = -\left(\frac{\varepsilon}{V}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3^-} \left(\frac{V_R}{Q}\right)
\] (5.14)

\textit{Extracellular phase}

\[
(1 - H_t) ([\text{HCO}_3^-]_{\text{pl, out}} - [\text{HCO}_3^-]_{\text{pl, in}}) = - H_t \left(\frac{\varepsilon}{V}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3^-} \left(\frac{V_R}{Q}\right)
\] (5.15)

\[
(1 - H_t) ([\text{Cl}^-]_{\text{pl, out}} - [\text{Cl}^-]_{\text{pl, in}}) = H_t \left(\frac{\varepsilon}{V}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3^-} \left(\frac{V_R}{Q}\right)
\] (5.16)

where

\([\text{HCO}_3^-]_{\text{rbc, out}} ([\text{HCO}_3^-]_{\text{rbc, in}})\) and \([\text{Cl}^-]_{\text{rbc, out}} ([\text{Cl}^-]_{\text{rbc, in}})\) are the intracellular concentrations of \(\text{HCO}_3^-\) and \(\text{Cl}^-\), respectively; subscripts "out" and "in" denote outlet and inlet compositions, respectively.

\([\text{HCO}_3^-]_{\text{pl, out}} ([\text{HCO}_3^-]_{\text{pl, in}})\) and \([\text{Cl}^-]_{\text{pl, out}} ([\text{Cl}^-]_{\text{pl, in}})\) are the extracellular concentrations of \(\text{HCO}_3^-\) and \(\text{Cl}^-\), respectively; subscripts "out" and "in" denote outlet and inlet compositions, respectively.

\(H_t\) is the hematocrit or the volume fraction of RBCs in the suspension.

\(\text{Flux}_{\text{HCO}_3^-} = \text{Function}\left\{k_{\text{trans}}, K_A; [\text{HCO}_3^-]_{\text{rbc}}, [\text{Cl}^-]_{\text{rbc}}, [\text{HCO}_3^-]_{\text{pl}}, [\text{Cl}^-]_{\text{pl}}\right\}\) or

\(\text{Flux}_{\text{HCO}_3^-} = \text{Function}'\left\{P_{\text{HCO}_3}, P_{\text{Cl}}, [\text{HCO}_3^-]_{\text{rbc}}, [\text{Cl}^-]_{\text{rbc}}, [\text{HCO}_3^-]_{\text{pl}}, [\text{Cl}^-]_{\text{pl}}\right\}\) which is given by either Equation (5.11) or (5.1), respectively.

\(V_R/Q\) is the residence time of the reactor where \(V_R\) is the volume of the CSTR and \(Q\) is the volumetric flowrate of the RBC suspension.
Table 5.4: Parameter values and objective function used in analysis of Klocke’s data.

<table>
<thead>
<tr>
<th>System parameters</th>
<th>$T = 37 , ^{\circ}C$, $Ht = 19% \text{ RBC}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC parameters</td>
<td>$(s/v)_{rbc} = 1.87 , \mu m^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$T_{tot} = 1 \times 10^6 \text{ transporters/RBC}$</td>
</tr>
</tbody>
</table>

Case 1: Chloride-loaded RBCs with bicarbonate-containing buffer @ pH 7.7

<table>
<thead>
<tr>
<th>Inlet concentrations (mM)</th>
<th>$[HCO_3^-]<em>{rbc} = 25.0$, $[Cl^-]</em>{rbc} = 125.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[HCO_3^-]<em>{pl} = 130.4$, $[Cl^-]</em>{pl} = 19.6$</td>
</tr>
<tr>
<td>Minimization objective function</td>
<td>$F(k_{trans, K}, \lambda) = \frac{\sum (</td>
</tr>
</tbody>
</table>

Case 2: Bicarbonate-loaded RBCs with chloride-containing buffer @ pH 7.7

<table>
<thead>
<tr>
<th>Inlet concentrations (mM)</th>
<th>$[HCO_3^-]<em>{rbc} = 146.0$, $[Cl^-]</em>{rbc} = 4.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[HCO_3^-]<em>{pl} = 20.5$, $[Cl^-]</em>{pl} = 129.5$</td>
</tr>
<tr>
<td>Minimization objective function</td>
<td>$F(k_{trans, K}, \lambda) = \frac{\sum (</td>
</tr>
</tbody>
</table>

5.2.2.2. Weith’s Experimental System (1979)

Counter-transport of $[^14]C$bicarbonate into human erythrocyte ghosts driven by a Cl$^-$ gradient was followed at a low cytocrit (volume fraction of ghosts in the suspension) of 1%, under the conditions where the amount of permeating anions in the ghosts is significant compared to the amount of permeating anions in the extracellular phase. Weith (1979) conducted the experiments in a stopped-flow rapid-reaction device at 0 $^\circ$C by having ghosts containing 165 mM KCl and 1 mM acetazolamide reacted with a Cl$^-$-free medium containing 4.2 $\mu$M H$^{14}$CO$_3^-$. As mentioned previously, his experiments utilized the fact that uncatalyzed rates of hydration/dehydration of carbonic acid proceed very slowly relative to the rate of anion exchange and isotopically labeled H$^{14}$CO$_3^-$ can be used to monitor HCO$_3^-$ movement. The decrease of extracellular H$^{14}$CO$_3^-$
concentration was followed by determinations of the extracellular radioactivity. The intracellular radioactivity was calculated from the disappearance of $\text{H}^{14}\text{CO}_3^-$ from the medium. It had been reported in the literature that the maximum transport capacity of anion transporter decreased by 10-15% when ghosts were stored at 0 °C for 24 hours (Funder and Weith, 1976). However, Weith's investigations were reported to be carried out with ghosts that were prepared on the day of the experiment; therefore $1 \times 10^6$ band 3 monomers per ghost is used in the simulation.

Because the transmembrane anion transport is, by comparison, a much slower process than the diffusion of anions in both the cell and extracellular medium, as a result, resistances to the diffusion of $\text{HCO}_3^-$ and $\text{Cl}^-$ in the intra- and extracellular boundary layers are neglected in this analysis. Weith's system is modeled as an ideal, isothermal batch reactor with two compartments; consequently, a simple model which consists a system of four simultaneous, nonlinear ordinary differential equations (ODEs) is obtained.

**Intracellular phase**

\[
\frac{d [\text{HCO}_3^-]_{\text{rbc}}}{dt} = \left(\frac{s}{v}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3} \tag{5.17}
\]

\[
\frac{d [\text{Cl}^-]_{\text{rbc}}}{dt} = -\left(\frac{s}{v}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3} \tag{5.18}
\]

**Extracellular phase**

\[
(1 - Ht) \frac{d [\text{HCO}_3^-]_{\text{pl}}}{dt} = -Ht \left(\frac{s}{v}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3} \tag{5.19}
\]

\[
(1 - Ht) \frac{d [\text{Cl}^-]_{\text{pl}}}{dt} = Ht \left(\frac{s}{v}\right)_{\text{rbc}} \text{Flux}_{\text{HCO}_3} \tag{5.20}
\]
where

\([HCO_3^-]_{rbc}\) and \([Cl^-]_{rbc}\) are the intracellular concentrations of \(HCO_3^-\) and \(Cl^-\), respectively.

\([HCO_3^-]_{pl}\) and \([Cl^-]_{pl}\) are the extracellular concentrations of \(HCO_3^-\) and \(Cl^-\), respectively.

Table 5.5: Parameter values and objective function used in analysis of Weith’s data.

<table>
<thead>
<tr>
<th>System parameters</th>
<th>(T = 0) °C, (pH = 8.7, Ht = 1%) ghost</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC parameters</td>
<td>((s/v)_{rbc} = 1.58 \mu m^{-1})†</td>
</tr>
<tr>
<td></td>
<td>(T_{tot} = 1 \times 10^6) transporters/ghost*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial concentrations</th>
<th>([HCO_3^-]<em>{rbc} = 0) M, ([Cl^-]</em>{rbc} = 165) mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([HCO_3^-]<em>{pl} = 4.2) \mu M, ([Cl^-]</em>{pl} = 0) M</td>
</tr>
</tbody>
</table>

Minimization objective function

\[
F(k_{trans}, K_\lambda) = \frac{\sum ((HCO_3^-)_{pl,exp} - (HCO_3^-)_{pl,calc})^2}{\sum ((HCO_3^-)_{pl,exp})^2} + \frac{\sum ((HCO_3^-)_{rbc,exp} - (HCO_3^-)_{rbc,calc})^2}{\sum ((HCO_3^-)_{rbc,exp})^2}
\]

† It was reported that ghost resealed with KCl had a membrane area of 1.42x10^-6 cm² per cell, and a cell volume of 90 \mu m³ (Funder and Weith, 1976).

* Weith et al.'s (1980) studies had demonstrated that the anion transport capacity of resealed ghosts is not hampered by the technique for preparing ghosts.

5.2.2.3 Illsley and Verkman’s Experimental System (1987)

Illsley and Verkman measured anion exchange using a Cl⁻ sensitive fluorescent probe which is 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ). It was reported that SPQ fluorescence was not altered by other physiological anions or \(pH\). The experiments were conducted by having sealed RBC ghosts which were loaded with 100 mM \(Cl^-\) and 1 mM SPQ reacted with isoosmotic \(Cl^-\)-free solutions containing either
HCO$_3^-$ or SO$_4^{2-}$ in a stopped-flow rapid-reaction apparatus at 37 °C. Addition of ghosts to the Cl$^-$-free medium caused a rapid increase in fluorescence as intracellular Cl$^-$ was exchanged for external HCO$_3^-$ and SPQ fluorescence was unquenched. Knowing the quenching constant of SPQ, initial and final Cl$^-$ concentrations and the corresponding fluorescences, one can then use the Stern-Volmer relationship to translate the fluorescence intensity to intracellular Cl$^-$ concentration. Equations (5.17) - (5.20) can be used to describe this system.

Table 5.6: Parameter values and objective function used in analysis of Illsley and Verkman’s data.

<table>
<thead>
<tr>
<th>System parameters</th>
<th>$T = 37$ °C, $Ht = 0.03%$ ghost</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC parameters</td>
<td>$(s/v)_{rbc} = 1.58 \mu m^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$T_{tot} = 1 \times 10^6$ transporters/ghost</td>
</tr>
</tbody>
</table>

Case 1: Chloride-loaded ghosts with bicarbonate-containing isoosmotic buffer

<table>
<thead>
<tr>
<th>Initial concentrations (mM)</th>
<th>$[HCO_3^-]<em>{rbc} = 0$, $[Cl^-]</em>{rbc} = 100$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[HCO_3^-]<em>{pl} = 20$, $[Cl^-]</em>{pl} = 0$</td>
</tr>
<tr>
<td>Minimization objective function</td>
<td>$F(k_{max}, K_A) = \sum (</td>
</tr>
<tr>
<td></td>
<td>$\sum (</td>
</tr>
</tbody>
</table>

Case 2: Chloride-loaded ghosts with sulfate-containing isoosmotic buffer

<table>
<thead>
<tr>
<th>Initial concentrations (mM)</th>
<th>$[SO_4^{2-}]<em>{rbc} = 0$, $[Cl^-]</em>{rbc} = 100$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[SO_4^{2-}]<em>{pl} = 66$, $[Cl^-]</em>{pl} = 0$</td>
</tr>
<tr>
<td>Minimization objective function</td>
<td>$F(k_{max}, K_A) = \sum (</td>
</tr>
<tr>
<td></td>
<td>$\sum (</td>
</tr>
</tbody>
</table>
5.2.2.4 Lemon's Experimental System (1989)

Lemon measured the pH changes of the Jacobs-Stewart cycle using a fluorescent indicator, pyranine (8-hydroxy-1,3,6-pyrene trisulfonic acid), in the extracellular medium in a stopped-flow mixing device at 25 °C. Special ion jump experiments, designed to minimize competition between Cl⁻ and HCO₃⁻, were performed by mixing 1 volume of essentially HCO₃⁻-free, Cl⁻-loaded packed cells with 14 volumes of various Cl⁻-free, HCO₃⁻-containing pyranine solutions. The following approach was implemented to formulate a model for describing Lemon's system:

1. After mixing, the RBCs were assumed to be uniformly distributed in the mixing cuvette of the stopped-flow device.

2. Extracellular resistance to CO₂ transport was taken into account with an estimated mass transfer coefficient. This transport resistance was included because CO₂ diffusion across the RBC membrane is a very rapid process in comparison to CO₂ diffusion through the unstirred layer which is a much slower process, perhaps one to two order magnitude slower depending on the \( P_{CO2} \) of the system. The magnitude of this external diffusion effect is estimated from a simple boundary layer analysis as given in Appendix B (Conn and Olson, 1979; Kagawa and Mochizuki, 1982; Vandegriff and Olson, 1984a and 1984b).

3. Since additional carbonic anhydrase enzyme was added to the extracellular medium, CO₂ hydration/dehydration reactions are assumed to be at chemical equilibrium both intra- and extracellularly.

4. Intra- and extracellular buffering capacities are taken into account. The dependence of extracellular buffering capacity on pH and \([HCO₃⁻]_{pl}\) is taken into consideration as shown in Equation (5.21) (Butler, 1964).

\[
\beta_{pl} = 2.303 \left[ \frac{K_{buffer}[buffer]_{pl}[H^+]_{pl}}{(K_{buffer} + [H^+]_{pl})^2} + \frac{K_{water}[H^+]_{pl}}{[H^+]_{pl}} \right] 
\]

(5.21)
where

$\beta_{pl}$ is the buffering capacity of the extracellular medium.

$K_{buffer}$ is the equilibrium constant of the buffer.

$[buffer]_{pl}$ is the molar concentration of the buffer in the extracellular medium.

$K_{water}$ is the dissociation constant of water.

The last two terms of Equation (5.21) are due to buffering effect of water; the first term is due to the conjugate acid base pair. In most cases of interest, the last two terms can be neglected. It should be mentioned that the contributions are additive; thus when there is a mixture of noninteractive buffers present in solution, their buffering effects are additive.

**Intracellular phase**

$$
\left(1 - \frac{\beta_{rbc}}{2.303[H^+]_{rbc}} \frac{d[H^+]_{rbc}}{d[HCO_3^-]_{rbc}}\right) \frac{d[HCO_3^-]_{rbc}}{dt} = \left(\frac{s}{v}\right)_{rbc} \text{Flux}_{HCO_3} \tag{5.22}
$$

$$
\frac{d[Cl^-]_{rbc}}{dt} = - \left(\frac{s}{v}\right)_{rbc} \text{Flux}_{HCO_3} \tag{5.23}
$$

$$
\left(1 + \frac{\beta_{rbc}}{2.303[H^+]_{rbc}} \frac{d[H^+]_{rbc}}{d[CO_2]_{rbc}}\right) \frac{d[CO_2]_{rbc}}{dt} = \left(\frac{s}{v}\right)_{rbc} \eta_{CO_2} \left(\frac{[CO_2]_{pl}}{\alpha_{CO_2,pl}} - \frac{[CO_2]_{rbc}}{\alpha_{CO_2,rbc}}\right) \tag{5.24}
$$

**Extracellular phase**

$$
(1 - Ht) \left(1 - \frac{\beta_{pl}}{2.303[H^+]_{pl}} \frac{d[H^+]_{pl}}{d[HCO_3^-]_{pl}}\right) \frac{d[HCO_3^-]_{pl}}{dt} = - Ht \left(\frac{s}{v}\right)_{rbc} \text{Flux}_{HCO_3} \tag{5.25}
$$

$$
(1 - Ht) \frac{d[Cl^-]_{pl}}{dt} = Ht \left(\frac{s}{v}\right)_{rbc} \text{Flux}_{HCO_3} \tag{5.26}
$$
\[(1 - Ht) \left(1 + \frac{\beta_{pl}}{2.303[H^+]_{pl}} \frac{d[H^+]_{pl}}{d^t[CO_2]_{pl}}\right) \frac{d[CO_2]_{pl}}{dt} = -Ht \left(\frac{\xi}{\nu} \right)_{rbc} \eta_{CO2} \frac{[CO_2]_{pl}}{\alpha_{CO2,pl}} \frac{[CO_2]_{rbc}}{\alpha_{CO2,rbc}}\]

where

\[
\frac{d[H^+]_{rbc}}{d[CO_3^-]_{rbc}} = - \frac{K' f_{water} [CO_2]_{rbc}}{([HCO_3^-]_{rbc})^2} \quad \text{and} \quad \frac{d[H^+]_{pl}}{d[CO_3^-]_{pl}} = - \frac{K' f_{water} [CO_2]_{pl}}{([HCO_3^-]_{pl})^2}.
\]

\[
\frac{d[H^+]_{rbc}}{d[CO_2]_{rbc}} = \frac{K' f_{water}}{[HCO_3^-]_{rbc}} \quad \text{and} \quad \frac{d[H^+]_{pl}}{d[CO_2]_{pl}} = \frac{K' f_{water}}{[HCO_3^-]_{pl}}.
\]

\(f_{water}\) is the fraction of water inside the RBC, and it is introduced here to account for the fact that concentrations inside the RBCs are expressed per unit cell volume instead per unit cell water volume.

\(\alpha_{CO2,rbc}\) and \(\alpha_{CO2,pl}\) are the solubility coefficients for CO\(_2\) in the RBC and the plasma, respectively.

\(\eta_{CO2}\) is the effective, lumped mass transfer coefficient for CO\(_2\); and its value is estimated in Appendix B.

**Table 5.7:** Parameter values and objective function used in analysis of Lemon's data.

<table>
<thead>
<tr>
<th>System parameters</th>
<th>(T = 25^\circ\text{C}, Ht = 6.7% \text{ RBC})</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC parameters</td>
<td>((s/\nu)_{rbc} = 1.87 \mu \text{m}^{-1})</td>
</tr>
<tr>
<td></td>
<td>(T_{tot} = 1\times10^6 \text{ transporters/RBC})</td>
</tr>
<tr>
<td></td>
<td>(f_{water} = 0.72, \beta_{rbc} = 0.057 \text{ M/pH})</td>
</tr>
<tr>
<td>Extracellular parameters</td>
<td>(\beta_{pl}) (calculated from Equation (5.21))</td>
</tr>
<tr>
<td>Physical properties of CO(_2)</td>
<td>(\alpha_{CO2,rbc} = 3.60\times10^{-5} \text{ M/mmHg})</td>
</tr>
<tr>
<td></td>
<td>(\alpha_{CO2,pl} = 4.05\times10^{-5} \text{ M/mmHg})</td>
</tr>
<tr>
<td>Mass transfer coefficient</td>
<td>(\eta_{CO2} = 6.0\times10^{-5} \text{ cm/sec/mmHg})</td>
</tr>
<tr>
<td>Equilibrium parameter</td>
<td>(p K' = 6.16)</td>
</tr>
</tbody>
</table>
Table 5.7: continued

<table>
<thead>
<tr>
<th>Case I: Chloride-loaded RBCs with buffer containing bicarbonate and pyranine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial concentrations (mM)</strong></td>
</tr>
<tr>
<td>(1) $[CO_2]<em>{rbc} = 0.116$, $[HCO_3^-]</em>{rbc} = 1.25$, $[Cl^-]_{rbc} = 21.6$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.161$, $[HCO_3^-]</em>{pl} = 3.52$, $[Cl^-]_{pl} = 5.22 \times 10^{-4}$</td>
</tr>
<tr>
<td>(2) $[CO_2]<em>{rbc} = 0.227$, $[HCO_3^-]</em>{rbc} = 3.23$, $[Cl^-]_{rbc} = 21.6$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.316$, $[HCO_3^-]</em>{pl} = 7.07$, $[Cl^-]_{pl} = 5.22 \times 10^{-4}$</td>
</tr>
<tr>
<td>Minimization objective function $F(k_{trans}, K_A) = \frac{\sum (\left[H^+\right]<em>{pl,exp} - \left[H^+\right]</em>{pl,calc})^2}{\sum \left[H^+\right]_{pl,exp}}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case II: Chloride-loaded RBCs with buffer containing bicarbonate and SPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial concentrations (mM)</strong></td>
</tr>
<tr>
<td>(1) $[CO_2]<em>{rbc} = 0.0137$, $[HCO_3^-]</em>{rbc} = 0.236$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.0190$, $[HCO_3^-]</em>{pl} = 0.330$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>(2) $[CO_2]<em>{rbc} = 0.0342$, $[HCO_3^-]</em>{rbc} = 0.583$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.0475$, $[HCO_3^-]</em>{pl} = 0.827$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>(3) $[CO_2]<em>{rbc} = 0.0683$, $[HCO_3^-]</em>{rbc} = 1.14$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.0949$, $[HCO_3^-]</em>{pl} = 1.60$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>(4) $[CO_2]<em>{rbc} = 0.340$, $[HCO_3^-]</em>{rbc} = 5.03$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.473$, $[HCO_3^-]</em>{pl} = 8.33$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>(5) $[CO_2]<em>{rbc} = 0.678$, $[HCO_3^-]</em>{rbc} = 8.85$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 0.941$, $[HCO_3^-]</em>{pl} = 16.7$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>(6) $[CO_2]<em>{rbc} = 1.35$, $[HCO_3^-]</em>{rbc} = 14.6$, $[Cl^-]_{rbc} = 50.4$</td>
</tr>
<tr>
<td>$[CO_2]<em>{pl} = 1.87$, $[HCO_3^-]</em>{pl} = 24.4$, $[Cl^-]_{pl} = 0.442$</td>
</tr>
<tr>
<td>Minimization objective function $F(k_{trans}, K_A) = \frac{\sum (\left[Cl^-\right]<em>{pl,exp} - \left[Cl^-\right]</em>{pl,calc})^2}{\sum \left[Cl^-\right]_{pl,exp}}$</td>
</tr>
</tbody>
</table>
5.2.3 Solution Methods

All the numerical computations are carried out on a Vax 2000 station. With all the parameters and inlet conditions being specified (Table 5.4), Equations (5.13) - (5.16) which is a set of nonlinear algebraic equations can be solved using a modification of the Powell hybrid method from MINPACK-1 (Moré et al., 1980) to give concentrations for various species as functions of reactor residence time. MINPACK-1 is a collection of efficient and robust optimization software. Entirely satisfactory global convergence results are available from the algorithms; and, in addition, the package’s properties allow scale invariant implementations. Along the same line, with all the parameters and initial conditions specified (Tables 5.5 and 5.6 and Table 5.7), Equations (5.17) - (5.20) and Equations (5.22) - (5.26), which are systems of simultaneous nonlinear ODEs, can be numerically integrated with EPISODE (Byrne et al., 1977) to give concentrations for various species as a function of reactor holding time. EPISODE is ideally suited for “stiff” differential equation systems (i.e., systems with widely varying time constants).

However, the goal here is to solve the inverse problem of characterizing $k_{trans}$ and $K_A$ (or $P_{HCO3}$ and $P_{Cl}$) by utilizing available experimental data in the literature. Therefore, the numerical procedure is initiated by input of the experimental measurements, immediately followed by the usage of a natural cubic spline to smooth the raw data and generate $[C_i]_{exp}$, where $C_i$ denotes all the species that are involved in a particular system. The next step is to supply the inlet or initial conditions and guesses for $k_{trans}$ and $K_A$ (or $P_{HCO3}$ and $P_{Cl}$). The material balance equations are then solved with the appropriate numerical method; and $k_{trans}$ and $K_A$ (or $P_{HCO3}$ and $P_{Cl}$) are then searched for by a nonlinear least squares fitting method. The nonlinear least squares fitting method searches for the values of both parameters that best fit the experimental data $[C_i]_{exp}$. In other words one seeks the values of $k_{trans}$ and $K_A$ (or
$P_{\text{HCO}_3}$ and $P_{Cl}$) which minimize the objective function $F(k_{\text{trans}}, K_A)$ (or $F(P_{\text{HCO}_3}, P_{Cl})$). The nonlinear function, $F(k_{\text{trans}}, K_A)$ (or $F(P_{\text{HCO}_3}, P_{Cl})$), is minimized using a non-gradient based minimization method based on the simplex method of Nelder and Meads (1965). This minimization algorithm has the advantages of simple calculations, uncomplicated logic and few adjustable parameters which need to be supplied. A major disadvantage of this algorithm is that the optimum values for the parameters may not represent a global minimum. To determine whether a set of parameters corresponds to a global minimum, different sets of initial guess can be used to initiate the search. The iterative procedure is continued until the error between the calculated and the experimental concentrations is sufficiently small. Numerical values for RBC/ghost parameters, inlet or initial conditions, and the minimization objective functions for various experimental systems used in the analysis are listed in Tables 5.4 - 5.7.

5.3 Results and Discussion

5.3.1 Comparison of the Simulation Results with the Experimental Data

5.3.1.a Comparison with Klocke's Data (1976)

Two sets of computations are performed for the experimental conditions given in Table 5.4. The first simulates the increase in extracellular $Cl^-$ concentration following mixture of $Cl^-$-loaded RBCs with bicarbonate buffer which is shown in Figure 5.6. The second computation simulates the change in extracellular $HCO_3^-$ content following mixture of $HCO_3^-$-loaded cells with chloride buffer which is given in Figure 5.7. In Figures 5.6 and 5.7, the solid lines represent the computed [Cl$^-$]$_{pl}$ and [HCO$_3^-$]$_{pl}$, respectively, versus residence time calculated using the flux expression based on the ping-pong model (Equation (5.11)) with $k_{\text{trans}}=4\times10^4$ sec$^{-1}$, $K_A=200$ M$^{-1}$ and $k_{\text{trans}}=6\times10^4$ sec$^{-1}$, $K_A=200$ M$^{-1}$, respectively. The dashed lines in the right-hand-side
Figure 5.6: Change in extracellular Cl\(^{-}\) concentration following mixture of Cl\(^{-}\)-loaded RBCs with HCO\(_3\)^{-}-containing buffer at 37 °C (more detail on the experimental conditions is given in Table 5.4). Klocke’s experimental results (Klocke, 1976) are represented as mean ± S.E. of the multiple analyses of each filtered sample. The left panel gives the comparison of simulation result generated using simplified ping-pong model with the experimental data. The theoretical line (——) is calculated with \(k_{\text{trans}}=4\times10^{4} \text{ sec}^{-1}\) and \(K_A=200 \text{ M}^{-1}\). The right panel gives the comparison of simulation result generated using the constant field electrodiffusion with phenomenological permeability coefficients with the experimental data. The theoretical curve (- - - -) is calculated assuming \(P_{\text{HCO}_3}=1.10\times10^{-3}\) and \(P_{\text{Cl}}=1.10\times10^{-4}\) cm/sec; (———), \(P_{\text{HCO}_3}=1.10\times10^{-4}\) and \(P_{\text{Cl}}=1.10\times10^{-4}\) cm/sec; (———), \(P_{\text{HCO}_3}=1.10\times10^{-5}\) and \(P_{\text{Cl}}=1.10\times10^{-4}\) cm/sec.
Figure 5.7: Change in extracellular HCO$_3^-$ concentration following mixture of HCO$_3^-$-loaded RBCs with Cl$^-$-containing buffer at 37 °C (more detail on the experimental conditions is given in Table 5.4). Klocke’s experimental results (Klocke, 1976) are represented as mean ± S.E. of the multiple analyses of each filtered sample. The left panel gives the comparison of simulation result generated using simplified ping-pong model with the experimental data. The theoretical line (———) is calculated with $k_{\text{trans}} = 6 \times 10^4$ sec$^{-1}$ and $K_A = 200$ M$^{-1}$. The right panel gives the comparison of simulation result generated using the constant field electrodiffusion with phenomenological permeability coefficients with the experimental data. The theoretical curve (-----) is calculated assuming $P_{\text{HCO3}} = 1.10 \times 10^{-3}$ and $P_{\text{Cl}} = 1.10 \times 10^{-4}$ cm/sec; (----), $P_{\text{HCO3}} = 1.10 \times 10^{-4}$ and $P_{\text{Cl}} = 1.10 \times 10^{-4}$ cm/sec; (-----), $P_{\text{HCO3}} = 1.10 \times 10^{-5}$ and $P_{\text{Cl}} = 1.10 \times 10^{-4}$ cm/sec.
panels are the computational results generated using flux expression derived based on constant field electrodiffusion (Equation (5.1)) using the range of permeability coefficients reported in the literature. These curves are calculated assuming $P_{Cl} = 1.1 \times 10^{-4} \text{ cm/sec}$ and $P_{HCO_3} = 1.1 \times 10^{-3} \text{ cm/sec}$ for the topmost of this group of curves, $P_{HCO_3} = 1.1 \times 10^{-4} \text{ cm/sec}$ for the middle curves, $P_{HCO_3} = 1.1 \times 10^{-5} \text{ cm/sec}$ for the bottom curves.

The experimental data points seem to indicate that 97% of reaction is complete in 0.4 sec, while simplified ping-pong model predicts 93% and constant filed electrodiffusion model predicts 91% of reaction is complete in 0.4 sec. As can be seen the agreement between the experimental data and the computational results generated by simplified ping-pong model and constant field passive diffusion theory (assuming $P_{HCO_3} = 1.1 \times 10^{-3} \text{ cm/sec}$ and $P_{Cl} = 1.1 \times 10^{-4} \text{ cm/sec}$) in the first 0.4 sec is good; both models, however, over predict the final concentrations. The simplified ping-pong model over predicts final $[Cl^-]_{pl}$ and $[HCO_3^-]_{pl}$ by 1.82 and 2.03 mM, respectively; and the constant field electrodiffusion model over predicts by 2.09 and 2.71 mM, respectively. The reason for this discrepancy is unclear; with the limited data it is difficult to sort out whether it is due to some experimental errors and/or artifacts or due to the fact that inhibitory effects on the anion transporter became significant at the later stage of the measurements which is not accounted for in the model. The algebraic deviations from the experimental data for the the curves generated by the simplified ping-pong equation are 0.10 mM in $[Cl^-]_{pl}$ and 0.17 mM in $[HCO_3^-]_{pl}$. On the other hand, the algebraic deviations for the curves computed by passive electrodiffusion equation are 0.16 mM in $[Cl^-]_{pl}$ and 0.29 mM in $[HCO_3^-]_{pl}$.

5.3.1.b Comparison with Weith’s Data (1979)

Figures 5.8 and 5.9 give the comparison of the simulation result generated using both models with Weith’s experimental data. The numerical values used for the
pertinent parameters and initial chemical compositions are given in Table 5.5. In Figure 5.8, the solid line represents the computed \([HCO_3^-]_{rbc}\) and \([HCO_3^-]_{pl}\) versus time by the simplified ping-pong model with \(k_{trans}=504\ \text{sec}^{-1}, K_A=390\ \text{M}^{-1}\). In Figure 5.9, the results are shown for different values of permeability coefficients, using the set \(P_{HCO_3}=3.8\times10^{-6}\) and \(P_{Cl}=1.7\times10^{-6}\ \text{cm/sec}\) which represents typical values reported in the literature for anion transport at 2 °C, and the set \(P_{HCO_3}=3.02\times10^{-5}\) and \(P_{Cl}=1.35\times10^{-5}\ \text{cm/sec}\) which represents the best-fitted values obtained in this study. From these figures, it is clear that excellent agreement is obtained between both model calculations and the experimental result. Although usage of the literature values for the permeability coefficients in the constant field electrodiffusion theory under estimates the transport by a substantial amount, a 10-fold increase of the permeabilities then give calculation that agrees closely with the experimental data.

It is noteworthy that after addition of Cl⁻-loaded ghosts to a Cl⁻-free, HCO₃⁻-containing medium, efflux of Cl⁻ was accompanied by an accumulation of HCO₃⁻ inside and depletion of medium HCO₃⁻ to give a final ratio of \([HCO_3^-]_{rbc}/[HCO_3^-]_{pl}=662\), this being very similar to the ratio of \([Cl^-]_{rbc}/[Cl^-]_{pl}=660\) at the end of experiment. Both flux expressions predict the final intracellular concentration is 647 times the extracellular concentration owing to the new Gibbs-Donnan equilibrium which is in agreement with the experimental observation. The maximum deviations between the results generated by the simplified ping-pong model and the experimental data are 14.4 \(\mu\text{M}\) in \([HCO_3^-]_{rbc}\) and 0.035 \(\mu\text{M}\) in \([HCO_3^-]_{pl}\); the algebraic deviations for both curves are 6.58 \(\mu\text{M}\) in \([HCO_3^-]_{rbc}\) and 0.015 \(\mu\text{M}\) in \([HCO_3^-]_{pl}\), respectively. On the other hand, the maximum deviations between the results simulated by the passive electrodiffusion model \((P_{HCO_3}=3.02\times10^{-5}\) and \(P_{Cl}=1.35\times10^{-5}\ \text{cm/sec})\) and the experimental data are 15.2 \(\mu\text{M}\) in \([HCO_3^-]_{rbc}\) and 0.066 \(\mu\text{M}\) in \([HCO_3^-]_{pl}\); the algebraic deviations for both curves are 7.38 \(\mu\text{M}\) in \([HCO_3^-]_{rbc}\) and 0.016 \(\mu\text{M}\) in \([HCO_3^-]_{pl}\), respectively.
Figure 5.8: Change in HCO$_3^-$ concentration inside and outside human RBC ghosts as a consequence of Cl$^-$/HCO$_3^-$ countertransport at 0 °C (more detail on the experimental conditions is given in Table 5.5). Intracellular HCO$_3^-$ concentrations are indicated on the left-hand ordinate, and extracellular concentrations on the right-hand ordinate; note difference of ordinate scales. Data points: experimental data from Weith (1978); filled circles for intracellular HCO$_3^-$ concentrations and open circles for extracellular HCO$_3^-$ concentrations. Curve: theoretical simulation of the experiment using the simplified ping-pong model; $k_{trans}$=504 sec$^{-1}$ and $K_A$=390 M$^{-1}$.
Figure 5.9: Change in $\text{HCO}_3^-$ concentration inside and outside human RBC ghosts as a consequence of $\text{Cl}^-/\text{HCO}_3^-$ countertransport at 0 °C (more detail on the experimental conditions is given in Table 5.5). Intracellular $\text{HCO}_3^-$ concentrations are indicated on the left-hand ordinate, and extracellular concentrations on the right-hand ordinate; note difference of ordinate scales. Data points: experimental data from Weith (1978); filled circles for intracellular $\text{HCO}_3^-$ concentrations and open circles for extracellular $\text{HCO}_3^-$ concentrations. Curve: theoretical simulation of the experiment using the constant field electrodiffusion with phenomenological permeability coefficients; (---) $P_{\text{HCO}_3}=3.02\times10^{-5}$ and $P_{\text{Cl}}=1.35\times10^{-5}$ cm/sec, and (--- for intracellular $\text{HCO}_3^-$, -- for extracellular for $\text{HCO}_3^-$) $P_{\text{HCO}_3}=3.80\times10^{-6}$ and $P_{\text{Cl}}=1.70\times10^{-6}$ cm/sec.
5.3.1.c  *Comparison with Illsley and Verkman’s Data* (1987)

Figures 5.10 and 5.11 show the comparison of the calculations obtained by both models and Illsley and Verkman’s (1987) red cell ghost chloride/anion exchange results; experimental conditions and relevant parameters for these cases are given in Table 5.6. Figure 5.10 illustrates the decrease in intracellular Cl⁻ concentration following mixture of Cl⁻-containing ghosts with bicarbonate buffer. Figure 5.11 demonstrates the change in intracellular Cl⁻ content following mixture of Cl⁻-loaded ghosts with K₂SO₄ buffer. In both figures, the solid lines represent the computed [Cl⁻]rbc versus time calculated using the simplified ping-pong model with \( k_{\text{trans}} = 4.5 \times 10^4 \text{ sec}^{-1} \), \( K_A = 200 \text{ M}^{-1} \) for the Cl⁻/HCO₃⁻ exchange; and \( k_{\text{trans}} = 400 \text{ sec}^{-1} \), \( K_A = 180 \text{ M}^{-1} \) for the Cl⁻/SO₄²⁻ exchange.

It is reported in the literature that anion transporter site has much lower affinity for SO₄²⁻ than for Cl⁻; i.e., \( K_{SO_4} \) is much smaller than \( K_{Cl} \) (Schnell *et al.*, 1977). Therefore, the transporter model developed in this work is not exactly appropriate for describing Cl⁻/SO₄²⁻ exchange due to the assumption that the association equilibrium constants are the same for both anions. A more appropriate model for considering Cl⁻/SO₄²⁻ exchange would be to relax this assumption and use \( k_{\text{trans}} = 4.5 \times 10^4 \text{ sec}^{-1} \), \( K_{Cl} = 200 \text{ M}^{-1} \) and \( K_{SO_4} = 2 \text{ M}^{-1} \) in the rederived flux expression.

The dashed lines in the right-hand-side panels are the computational results generated using the phenomenological permeability coefficients. In Figure 5.10, the results are shown for different values of permeability coefficients, using the set \( P_{HCO_3} = 1.1 \times 10^{-3} \) and \( P_{Cl} = 1.1 \times 10^{-4} \text{ cm/sec} \) which represents typical values reported in the literature for anion transport at 37 °C, and the set \( P_{HCO_3} = 1.58 \times 10^{-3} \) and \( P_{Cl} = 4.6 \times 10^{-4} \text{ cm/sec} \) which represents the best-fitted values obtained in this study. In Figure 5.11, the dashed line is obtained by using \( P_{HCO_3} = 2.4 \times 10^{-6} \) and \( P_{SO_4} = 2.95 \times 10^{-6} \text{ cm/sec} \) which represents the set of permeabilities that gives the best-fitted result.
Figure 5.10: Change in intracellular Cl\(^-\) concentration following mixture of Cl\(^-\)-loaded RBCs with a Cl\(^-\)-free, HCO\(_3\)^-containing buffer at 37 °C (more detail on the experimental conditions is given in Table 5.6). Illsley and Verkman’s experimental results (Illsley and Verkman, 1987) are represented as filled circles. The left panel gives the comparison of simulation result generated using simplified ping-pong model with the experimental data. The theoretical line (———) is calculated with \(k_{\text{trans}}=4.5\times10^4\) sec\(^{-1}\) and \(K_A=200\) M\(^{-1}\). The right panel gives the comparison of simulation result generated using the constant field electrodiffusion with phenomenological permeability coefficients with the experimental data. The theoretical curve (---) is calculated assuming \(P_{\text{HCO}_3}=1.58\times10^{-3}\) and \(P_{\text{Cl}}=4.60\times10^{-4}\) cm/sec; (---), \(P_{\text{HCO}_3}=1.10\times10^{-3}\) and \(P_{\text{Cl}}=1.10\times10^{-4}\) cm/sec.
Figure 5.11: Change in intracellular Cl⁻ concentration following mixture of Cl⁻-loaded RBCs with a Cl⁻-free, SO₄²⁻-containing buffer at 37 °C (more detail on the experimental conditions is given in Table 5.6). Illsley and Verkman's experimental results (Illsley and Verkman, 1987) are represented as filled circles. The left panel gives the comparison of simulation result generated using simplified ping-pong model with the experimental data; the theoretical line (-----) is calculated with $k_{\text{trans}}=400$ sec⁻¹ and $K_A=180$ M⁻¹. The right panel gives the comparison of simulation result generated using the constant field electrodiffusion with phenomenological permeability coefficients with the experimental data; the theoretical curve (- - - -) is calculated assuming $P_{SO_4}=2.40\times10^{-6}$ and $P_{Cl}=2.95\times10^{-6}$ cm/sec.
The maximum deviations between the calculations generated by the simplified ping-pong model and the experimental data are 4.87 mM and 3.67 mM in $[Cl^-]_{rbc}$ for cases 1 and 2, respectively; the algebraic deviations for these curves are 0.505 mM and 1.23 mM in $[Cl^-]_{rbc}$ for cases 1 and 2, respectively. On the other hand, the maximum deviations between the result calculated by the passive electrodiffusion model and the experimental data are 13.0 mM and 4.2 mM in $[Cl^-]_{rbc}$ for case 1 and 2, respectively; the algebraic deviations for these curves are 2.04 mM and 0.61 mM in $[Cl^-]_{rbc}$ for cases 1 and 2, respectively. As can be seen the agreement between the experimental data and the computational results generated by simplified ping-pong model is slightly better than that calculated by constant field electrodiffusion model.

5.3.1.d  Comparison with Lemon’s Data (1989)

Figures 5.12 and 5.13 show the extracellular pH, and Figures 5.14 - 5.19 show the extracellular Cl\textsuperscript– concentration computed by the simplified ping-pong (solid line) and passive electrodiffusion models (dashed line) for the experimental conditions listed in Table 5.7, with the experimentally determined time courses of extracellular pH or Cl\textsuperscript– concentration from the stopped-flow experiments of Lemon (1989) which are redrawn here for comparison. The ping-pong simulations agree well with the experimental data with the values of $k_{trans} = 1.1 \times 10^4$ sec\textsuperscript–1, $K_A = 200$ M\textsuperscript–1. On the other hand, the calculations obtained by using constant values of $P_{HCO_3}$ and $P_{Cl}$ (both have values of $3.16 \times 10^{-4}$ cm/sec which represent the best-fitted permeability coefficients) over estimate the observed transport rates by a noticeable amount. The maximum deviations between the results generated by the simplified ping-pong model and the experimental data are 0.494 nM in $[H^+]_{pl}$ and 0.129 mM in $[Cl^-]_{pl}$; the algebraic deviations for both curves are 0.276 nM in $[H^+]_{pl}$ and 0.11 mM in $[Cl^-]_{pl}$, respectively. On the other hand, the maximum deviations between the results simulated by the passive electrodiffusion model and the experimental data are 1.00 nM in $[H^+]_{pl}$ and 0.903 mM in $[Cl^-]_{pl}$; the algebraic
Figure 5.12: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case I.1). The chloride jump pH time course was collected by mixing Cl⁻-loaded RBCs with a Cl⁻-free 200 µM pyranine solution. Data: experimental trace of the extracellular pH from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with $k_{trans}=1.1 \times 10^4$ sec$^{-1}$ and $K_A=200$ M$^{-1}$, and (- - - - -) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16 \times 10^{-4}$ and $P_{Cl}=3.16 \times 10^{-4}$ cm/sec.
Figure 5.13: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case I.2). The chloride jump pH time course was collected by mixing Cl⁻-loaded RBCs with a Cl⁻-free 200 μM pyranine solution. Data: experimental trace of the extracellular pH from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with $k_{trans}=1.1\times10^4$ sec⁻¹ and $K_A=200$ M⁻¹, and (---) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16\times10^{-4}$ and $P_{Cl}=3.16\times10^{-4}$ cm/sec.
Figure 5.14: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.1). The chloride jump Cl⁻ time course was collected by mixing Cl⁻-loaded RBCs with a HCO₃⁻-containing 220 µM SPQ solution. Data: experimental trace of the extracellular Cl⁻ concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with $k_{\text{trans}}=1.1\times10^4$ sec⁻¹ and $K_A=200$ M⁻¹, and (-----) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16\times10^{-4}$ and $P_{Cl}=3.16\times10^{-4}$ cm/sec.
Figure 5.15: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.2). The chloride jump Cl⁻ time course was collected by mixing Cl⁻-loaded RBCs with a HCO₃⁻-containing 220 µM SPQ solution. Data: experimental trace of the extracellular Cl⁻ concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (-----) the simplified ping-pong model with $k_{\text{trans}}=1.1\times10^4$ sec⁻¹ and $K_A=200$ M⁻¹, and (- - - - -) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16\times10^{-4}$ and $P_{Cl}=3.16\times10^{-4}$ cm/sec.
**Figure 5.16**: Comparison of models for kinetics of Cl\(^-\)/HCO\(_3\)^- exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.3). The chloride jump Cl\(^-\) time course was collected by mixing Cl\(^-\)-loaded RBCs with a HCO\(_3\)^- containing 220 μM SPQ solution. Data: experimental trace of the extracellular Cl\(^-\) concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (——) the simplified ping-pong model with \(k_{\text{trans}}=1.1 \times 10^4\) sec\(^{-1}\) and \(K_A=200\) M\(^{-1}\), and (----) the constant field electrodiffusion with phenomenological permeability coefficients \(P_{\text{HCO3}}=3.16 \times 10^{-4}\) and \(P_{\text{Cl}}=3.16 \times 10^{-4}\) cm/sec.
Figure 5.17: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.4). The chloride jump Cl⁻ time course was collected by mixing Cl⁻-loaded RBCs with a HCO₃⁻-containing 220 µM SPQ solution. Data: experimental trace of the extracellular Cl⁻ concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with $k_{irans}=1.1\times10^4$ sec⁻¹ and $K_A=200$ M⁻¹, and (---) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16\times10^{-4}$ and $P_C=3.16\times10^{-4}$ cm/sec.
Figure 5.18: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.5). The chloride jump Cl⁻ time course was collected by mixing Cl⁻-loaded RBCs with a HCO₃⁻-containing 220 μM SPQ solution. Data: experimental trace of the extracellular Cl⁻ concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with $k_{trans}=1.1 \times 10^4$ sec⁻¹ and $K_A=200$ M⁻¹, and (- - - - -) the constant field electrodiffusion with phenomenological permeability coefficients $P_{HCO_3}=3.16 \times 10^{-4}$ and $P_{Cl}=3.16 \times 10^{-4}$ cm/sec.
Figure 5.19: Comparison of models for kinetics of Cl⁻/HCO₃⁻ exchange at 25 °C (more detail on the experimental conditions is given in Table 5.7, case II.6). The chloride jump Cl⁻ time course was collected by mixing Cl⁻-loaded RBCs with a HCO₃⁻-containing 220 µM SPQ solution. Data: experimental trace of the extracellular Cl⁻ concentration from Lemon (1989). Curves: theoretical simulations of the experiment; (———) the simplified ping-pong model with \( k_{\text{trans}} = 1.1 \times 10^4 \) sec\(^{-1}\) and \( K_A = 200 \) M\(^{-1}\), and (- - - - -) the constant field electrodiffusion with phenomenological permeability coefficients \( P_{HCO_3} = 3.16 \times 10^{-4} \) and \( P_{Cl} = 3.16 \times 10^{-4} \) cm/sec.
deviations for both curves are 0.571 nM in \([H^+]_{pl}\) and 0.290 mM in \([Cl^-]_{pl}\), respectively. As can be seen the agreement between the experimental data and the computational results generated by simplified ping-pong model is better than that calculated by constant field electrodiffusion model.

5.3.2 Comparison of the Determined Kinetic Parameters to Previous Determinations

Kinetic parameters used for the best-fitted simplified ping-pong simulations for various experimental systems are listed in Table 5.8. Literature values for \(K_A\) are difficult to come by because most of the binding constants reported in the literature are “apparent” values which depend strongly on the experimental conditions. As Lemon (1989) pointed out, a better estimate of the intrinsic binding rate constant comes from studies of competition between anions. Lemon’s argument was that by definition a competitive inhibitor binds to the transport site, and the point at which the transport rate for a given substrate is halved by a competitive anion should define the affinity of the site for the competing anion. The competitive inhibition binding constant for \(HCO_3^-\) competing against extracellular \(Cl^-\) in \(Cl^-/HCO_3^-\) exchange at 0 °C was reported to be in the range of 167 - 333 M\(^{-1}\) (Gunn et al., 1973). The competitive inhibition binding constant for \(Cl^-\) competing with the binding of the specific anion transport inhibitor was reported to be 190 M\(^{-1}\) at 20 °C (Frohlich and Gunn, 1986). In addition, a value of 200 M\(^{-1}\) was reported for the competitive inhibition binding constant for \(HCO_3^-\) at 37 °C (Lambert and Lowe, 1980). Finally, it should be mentioned that Lemon’s analysis on his own data which involved a different mathematical formulation of ping-pong model also indicated that usage of \(k_{trans}=1.1x10^4\) sec\(^{-1}\), \(K_{HCO3}=K_{Cl}=200\) M\(^{-1}\) did result in a more satisfactory fit between experimental data and model calculations. Therefore, it is
seen that the agreement between the $K_A$ values estimated in this work and the literature values for different temperatures is excellent.

Estimates for $k_{trans}$ in the literature come from Brahm's (1977) calculations of the anion transporter turnover number and Falke et al.'s (1985) NMR studies on the transport cycle. Using his data for Cl$^-$/Cl$^-$ exchange, Brahm (1977) calculated a turnover number of $1.3 \times 10^4$ sec$^{-1}$ at 25 °C and $5 \times 10^4$ sec$^{-1}$ at 37 °C. Because the translocation step is the rate-limiting step, the translocation rate must approximately equal the turnover rate of the transport cycle. Falke et al.'s NMR data specified that the translocation rate constant is $\approx 400$ sec$^{-1}$ at 0 °C and $\approx 8 \times 10^4$ sec$^{-1}$ at 37 °C (see Figure 5.3). Again, the $k_{trans}$ values determined in this analysis and reported literature values are in good agreement for different temperatures.

**Table 5.8:** Kinetic parameters obtained for Cl$^-$/HCO$_3^-$ exchange across the RBC membrane from analyzing different workers' experimental data using Equation (5.11).

<table>
<thead>
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<th>$k_{trans}$ (sec$^{-1}$)</th>
<th>$K_A$ (M$^{-1}$)</th>
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<tbody>
<tr>
<td>Exchange at 0 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weihl; Cl$^-$/HCO$_3^-$</td>
<td>504</td>
<td>390</td>
</tr>
<tr>
<td>Exchange at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon; Cl$^-$/HCO$_3^-$</td>
<td>$1.1 \times 10^4$</td>
<td>200</td>
</tr>
<tr>
<td>Exchange at 37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klocke; Cl$^-$/HCO$_3^-$</td>
<td>$4.6 \times 10^4$</td>
<td>200</td>
</tr>
<tr>
<td>Ilsley and Verkman;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl$^-$/HCO$_3^-$</td>
<td>$4.5 \times 10^4$</td>
<td>200</td>
</tr>
</tbody>
</table>
Permeability coefficients used for the best-fitted constant field electrodiffusion computations for various experimental systems are listed in Table 5.9. With the usage of permeabilities which are reported in the literature (Table 5.1), the phenomenological electrodiffusion theory generates the predictions that are sometimes in poor agreement with the observed rate of transport. For instance, the best-fitted result for Weith's system is achieved using permeabilities which are an order of magnitude higher than the reported literature values. Although the flux predicted by the constant field equation fits the data adequately, there are some discrepancies between the best-fitted values for $P_{HCO3}$ and $P_{Cl}$ obtained in this study and the literature values. It is observed that there is a wide variation of permeability coefficients reported both in the literature and in this study. This is because the concentrations of Cl$^-$ and HCO$_3^-$ were varied experimentally over a wide range. In some cases, the carrier is saturated. In others, the processes are like permeation and effectively bimolecular or diffusion driven.

Table 5.9: Best-fitted permeability coefficients obtained for Cl$^-$/HCO$_3^-$ exchange across the RBC membrane from analyzing different workers' experimental data using Equation (5.1).

<table>
<thead>
<tr>
<th></th>
<th>$P_{HCO3}$ (cm/sec)</th>
<th>$P_{Cl}$ (cm/sec)</th>
</tr>
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<tbody>
<tr>
<td>Exchange at 0 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weith; Cl$^-$/HCO$_3^-$</td>
<td>3.02x10$^{-5}$</td>
<td>1.35x10$^{-5}$</td>
</tr>
<tr>
<td>Exchange at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon; Cl$^-$/HCO$_3^-$</td>
<td>3.16x10$^{-4}$</td>
<td>3.16x10$^{-4}$</td>
</tr>
<tr>
<td>Exchange at 37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klocke; Cl$^-$/HCO$_3^-$</td>
<td>1.10x10$^{-3}$</td>
<td>1.10x10$^{-4}$</td>
</tr>
<tr>
<td>Illsley and Verkman;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl$^-$/HCO$_3^-$</td>
<td>1.58x10$^{-3}$</td>
<td>4.60x10$^{-4}$</td>
</tr>
</tbody>
</table>
5.4 Summary

Most of data on univalent anion movement across the RBC membrane have been interpreted by the use of a constant field diffusion of ion down an electrochemical gradient, slightly modified in the sense that phenomenological permeability coefficients were introduced to replace the ion mobilities in Goldman's solution. Although with appropriate values of $P_{HCO_3}$ and $P_{Cl}$, the passive electrodiffusion model generates simulation results which are reasonably compatible with some of the experimental data in the literature, this interpretation of anion exchange has been questioned because of the numerous evidences which indicate that anion exchange is mediated by a carrier mechanism. Furthermore, the derivation of Goldman's equation for ion fluxes and the membrane potential is not free of contradictions. On the one hand, one must assume that the membrane is so thin that the electrical potential profile may be considered as linear; on the other hand, integration of the differential equation across the thickness of the membrane should be physically meaningful. This inconsistency itself already circumscribes the validity of Equation (5.1). Therefore, the computed permeability values are only "effective" and must be interpreted accordingly.

Because the mechanism is dependent on carrier mediation which proceeds with obligatory exchange of an electrically neutral complex, the simplified ping-pong model is theoretically a more appropriate framework for analysis of anion transport data. Despite the assumptions which were introduced to simplify the model, the simplified ping-pong scheme appears to be of sufficient accuracy for describing the anion exchange. It is important to realize that while the curve-fitting technique used to obtain the kinetic parameters is accurate, its usefulness is limited by both the experimental error and the validity of the model. If the model comes close in representing the actual physical situation, then proper estimation of the kinetic parameters becomes possible.
when accurate experimental data is available. In addition, these parameters are not phenomenological but true intrinsic properties.

As mentioned previously, it is difficult to reconcile the fundamental observations and concepts on anion exchange kinetics with the notion of electrodiffusion of aqueous soluble anions. The simplified ping-pong model is both on a more satisfactory theoretical basis, and consistently provides better agreement with the available experimental data. Therefore, the conclusion of present work is that simplified ping-pong model has substantial advantages over the constant-field electrodiffusion model in the mathematical simulation of anion exchange.
CHAPTER 6

A THEORETICAL MODEL FOR GAS TRANSPORT AND ACID/BASE REGULATION BY BLOOD FLOWING IN MICROVESSELS

As has already been mentioned, the binding of $O_2$ at one hemoglobin site is coupled with binding of other substances at other sites due to conformational change of the hemoglobin molecule. In particular, $O_2$ binding to hemoglobin is affected by $P_{CO_2}$, $pH$, $[DPG]$ and other factors; and, in turn, $O_2$ binding affects transport of other substances, such as $CO_2$ and $H^+$. Thus a more complete description of gas transport can be achieved by considering simultaneous interactions between hemoglobin, $O_2$, $CO_2$, $H^+$, and other intermediate compounds. $CO_2$ exchange involves multiple interrelated physical and chemical events as depicted in Figure 2.1. Although there is a great deal of data dealing with the kinetics of individual events in $CO_2$ exchange, due to the fact that these processes are entwined in a complex manner, there have been limited attempts in studying the integrated system as a whole by incorporating the complete set of chemical and physical events into a computational model. Most of the work thus far in computational models which aimed at providing insights into the coupled $O_2$ and $CO_2$ transfer have been achieved by a semi-empirical, lumped-parameter approach.

Due to the complex nature of an exact transport model, various simplifications were introduced by previous investigators in order to develop tractable theoretical analyses. An adequate theory for the $O_2$ and $CO_2$ transfer problem has not been developed and verified for a wide range of tube diameters. Existing theories which treated blood as a homogeneous fluid and assumed local chemical equilibrium have been validated to some extent for macrochannel devices with diameters of several
hundred $\mu$m, but not for small microvessels. In addition, the interdependence of all species had been represented by $O_2$ and $CO_2$ dissociation curves. Theoretical developments along this line neglected not only the synergism among various species in the blood but also posed some problems in the interpretation of the effective, lumped properties. From the $O_2$ transport studies, it is observed that continuum models become less satisfactory for describing the transport in small microvessels where the discrete nature of blood begins to play a more important role. Therefore, there is a question on the validity of the continuum assumption for $CO_2$ transport when the flow channel dimension and RBC diameter are of comparable size. A local chemical equilibrium assumption is expected to be a good approximation for describing the chemical behavior of intracellular $CO_2$ due to the presence of carbonic anhydrase. However, this assumption is likely not appropriate for describing extracellular $CO_2$ hydration/dehydration reactions.

This work aims at utilizing available literature data which deals with the rate of individual events in $O_2$ and $CO_2$ exchange for the development of a mathematical model which is discrete in nature and takes into account the important physiological and biochemical processes underlying gas transport and $pH$ regulation by flowing blood. Experimental validation of the model is necessary; however, only few works in the literature have been accompanied with data that included measured total $CO_2$ transfer rates. Additionally, these experimental data were obtained for vessels whose diameters are several hundred $\mu$m and larger. No data is yet available for small vessels whose diameters are less than 100 $\mu$m. As a result, critical validation of this mathematical model over a wide range of diameters is still not possible. In this study, Voorhees' quantitative data on simultaneous $O_2$ and $CO_2$ transfer to and from blood flowing in tubular membrane oxygenators with diameters of $\approx 1.5$ mm (Voorhees,
1976) is used to check on the performance of this mathematical model in this very large diameter regime.

In addition to the development and limiting-case validation of the computational model, this analysis is used to address several specific questions. The first pertains to the incorporation of the coupled CO₂ transport by blood in relationship to O₂ transport in small microvessels with diameters of about 27 μm. Many previous analyses of gas transport by blood flowing in microvessels have been concerned with the O₂ transport problem alone. As a result, the effects of change in O₂ affinity as a result of pH and P_{CO₂} variations and the acid/base balance in blood were not evaluated by those models. Therefore, an O₂ transport model is evaluated in comparison to the more complete O₂/CO₂ exchange model to assess the error incurred by neglect of CO₂ transport effects. Often in prior model studies if CO₂ transport was considered, the description was simplified by treating blood as a continuum with the transferring species in local chemical equilibrium. Thus, the second question relates to the significance of those simplifications. The third question pertains to the effects of RBC HCO₃⁻/Cl⁻ exchange kinetics on CO₂ transfer. It has been suggested that under certain circumstances the transfer of CO₂ is limited by the rate of anion exchange across the RBC membrane (Weith and Brahm, 1980; Crandall and Bidani, 1981; Crandall et al., 1981). Finally, a study is made to determine the regime of applicability of the continuum and discrete models.

6.1 Development of Mathematical Model

6.1.1 Physical Situation

Before preceding to derive the necessary equations for the gas transport model, the chemistry and kinetics of the reactions will be considered. The reactions and
transport of O₂ and CO₂ in blood are complex phenomena. The scheme illustrated in Figure 2.1 is a simplified representation of the actual system. However, Figure 2.1 is a justifiable depiction of the system for the reasons discussed below. The following reactions are known to be taking place in both the RBC and plasma phases.

\[
\begin{align*}
CO₂ + H₂O &\rightleftharpoons H₂CO₃ \rightleftharpoons H^+ + HCO₃^- \quad (6.1a) \\
CO₂ + OH^- &\rightleftharpoons HCO₃^- \rightleftharpoons H^+ + CO₃^{2-} \quad (6.1b) \\
H₂O &\rightleftharpoons H^+ + OH^- \quad (6.1c) \\
H₃PO₄ &\rightleftharpoons H₂PO₄^- + H^+ \quad (6.1la) \\
H₂PO₄^- &\rightleftharpoons HPO₄^{2-} + H^+ \quad (6.1lb) \\
HPO₄^{2-} &\rightleftharpoons PO₄^{3-} + H^+ \quad (6.1lc) \\
NaHCO₃ &\rightleftharpoons Na^+ + HCO₃^- \quad (6.1IIa) \\
NaCl &\rightleftharpoons Na^+ + Cl^- \quad (6.1IIb) \\
KCl &\rightleftharpoons K^+ + Cl^- \quad (6.1IIIc)
\end{align*}
\]

Reactions (6.1a) - (6.1c) and (6.1IIa) are typical of reactions of CO₂ in aqueous media. However, at physiological pH the contribution to the formation of HCO₃⁻ via Reaction (6.1b) is small, and negligible amount of carbonate ion (CO₃^{2-}) is formed relative to the amounts of dissolved CO₂ and HCO₃⁻ present; as a result, Reaction (6.1b) is neglected. Although blood contains other buffer systems, such as phosphates (Reactions (6.1la) - (6.1lc)), their effects are minimal due to their small concentrations. Ionic species, such as Na⁺ and K⁺, are not involved in any important reactions but in
maintaining electroneutrality; nevertheless, it is necessary to account for their compositions in the medium when estimating the diffusivities of $\text{H}^+$, $\text{HCO}_3^-$ and $\text{Cl}^-$.

Inside the RBC, Reactions (6.I) - (6.III) and Reactions (6.IV) - (6.VII) are known to take place.

\[
K_z
\]
\[
\text{HbNH}_3^+ \iff \text{HbNH}_2 + \text{H}^+
\quad (6.\text{IV})
\]

\[
k
\]
\[
\text{HbNH}_2 + \text{O}_2 \iff \text{HbNH}_2\text{O}_2
\]

\[
k'\]

\[
k_a \quad K_a
\]
\[
\text{HbNH}_2 + \text{CO}_2 \iff \text{HbNHCOOH} \iff \text{HbNHCO}^- + \text{H}^+
\quad (6.\text{VI})
\]

\[
k_d\]

\[
k_a' \quad K_a'
\]
\[
\text{HbNH}_2\text{O}_2 + \text{CO}_2 \iff \text{O}_2\text{HbNHCOOH} \iff \text{O}_2\text{HbNHCO}^- + \text{H}^+
\quad (6.\text{VII})
\]

where

$K_z$ is the amino group ionization constant for the reduced hemoglobin, (Reaction (6.IV)).

$k$ and $k'$ are the dissociation and association rate constants of Reaction (6.V), respectively; and $k' = \frac{k}{[\text{O}_2]_{\text{rec}}} \left(\frac{P_{\text{O}_2}}{P_{50}}\right)^n$ according to the one-step variable rate coefficient method (Moll, 1969; Clark et al., 1985; Vandegriff and Olson, 1984b).

$K_a$ and $K_a'$ are the amino group ionization constants for deoxygenated and oxygenated hemoglobin carbamate, respectively (Reactions (6.VI) and (6.VII)).
$k_a$ and $k'_a$ are the hemoglobin-CO$_2$ reaction forward rate constants for Reactions (6.VI) and (6.VII), respectively.

$k_d$ and $k'_d$ are the hemoglobin-CO$_2$ reaction reverse rate constants for Reactions (6.VI) and (6.VII), respectively.

In the plasma, Reactions (6.I) - (6.III) and Reaction (6.VIII) are known to be taking place.

\[ RNH_3^+ \Leftrightarrow RNH_2 + H^+ \]  \hspace{1cm} (6.VIIIa)

\[ RNH_2 + CO_2 \Leftrightarrow RNHCONH \Leftrightarrow RNHCOO^- + H^+ \]  \hspace{1cm} (6.VIIIb)

Reaction (6.VIII) represents the reaction of CO$_2$ with proteins, amino acids and aliphatic aminos contained in the plasma. However, the carbamate or carbamino compounds that arise from combination of CO$_2$ with plasma proteins have a much smaller effect as compared to carbamino hemoglobin because of the relatively unfavorable equilibria for their formation. Consequently, the formation of plasma carbamate is neglected, but the buffering action of the plasma proteins is taken into account.

It should also be mentioned that RBC membrane is relatively impermeable to cations and that there are three anionic species that move across the RBC membrane which are potentially important in gas exchange - HCO$_3^-$, Cl$^-$ and OH$^-$. Because the concentration of OH$^-$ is extremely low relative to HCO$_3^-$ and Cl$^-$, the corresponding flux of OH$^-$ across the RBC membrane is small and negligible (Crandall et al., 1971). Furthermore, since water vapor pressure varies with osmolality, transfer of water is inevitable. As RBCs pass through the lung, HCO$_3^-$ undergoes CO$_2$ hydration reaction, and Cl$^-$ moves out the RBCs; this leads to a decrease in osmolality which causes water
to move out. At the venous, the opposite situation occurs, and RBCs take up water. However, this effect is not significant because there is only 2-3% difference in the hematocrit of arterial and venous blood. As a result, transfer of OH\textsuperscript{-} and water across the RBC membrane is neglected in this analysis.

6.1.2 Transport Equations

This model is an extension of the previous O\textsubscript{2} transport model which considered only reactions (2) and (3) in Figure 2.1. In the present model additional complexities, namely CO\textsubscript{2} transport and pH regulation by blood, are incorporated. As in the previous model, the RBC suspension is treated as two continuous, coexisting phases consisting of RBCs and plasma. The microvessel is divided into two regions: the central RBC-rich plasma region and the outer, RBC-free region. Radial distribution of RBCs and flow velocity profiles of RBCs and plasma are included. A description of the events shown in Figure 2.1 plus Reaction (6.77) can be obtained by making mass balances for each of the chemical species involved. These include oxygen, oxyhemoglobin, carbon dioxide, hemoglobin carbamate and hydrogen, bicarbonate and chloride ions. Since some of these species exist at different concentrations in the intra- and extracellular compartments, the behavior of 18 chemical species are described as a function of \( r \) and \( z \). The mass balances describing the change of each of the 18 species involve (a) transport of the species due to bulk convection of both the RBCs and plasma and radial diffusion in the plasma, (b) rate of consumption or production of that species by chemical reaction within its compartments, and/or (c) net transport of some of the species into or out of its compartment. The equations defining the analysis are developed below.
RBC Phase (0 ≤ r ≤ r_r)

The equations of continuity for the various species involved inside the RBCs are:

\[ V_{rbc}(r) \frac{\partial [O_2]_{rbc}}{\partial z} = -\left( \frac{s}{\nu_{rbc}} \right) \text{Flux}_{O_2} - R_{\text{HbO}_2} \]  \hspace{1cm} (6.1)

\[ V_{rbc}(r) \frac{\partial [HbO_2]_{rbc}}{\partial z} = R_{\text{HbO}_2} - R_{\text{O}_2\text{HbCO}_2} \]  \hspace{1cm} (6.2)

\[ V_{rbc}(r) \frac{\partial [CO_2]_{rbc}}{\partial z} = \left( \frac{s}{\nu_{rbc}} \right) \text{Flux}_{CO_2} - R_{\text{HCO}_3, rbc} - R_{\text{HbCO}_2} - R_{\text{O}_2\text{HbCO}_2} \]  \hspace{1cm} (6.3)

\[ V_{rbc}(r) \frac{\partial [HbCO_2]_{rbc}}{\partial z} = R_{\text{HbCO}_2} \]  \hspace{1cm} (6.4)

\[ V_{rbc}(r) \frac{\partial [O_2HbCO_2]_{rbc}}{\partial z} = R_{\text{O}_2\text{HbCO}_2} \]  \hspace{1cm} (6.5)

\[ V_{rbc}(r) \frac{\partial [H^+]_{rbc}}{\partial z} = \left( R_{\text{HCO}_3, rbc} + \sigma_1 R_{\text{HbO}_2} + \sigma_2 R_{\text{HbCO}_2} + \sigma_3 R_{\text{O}_2\text{HbCO}_2} \right) \left( \frac{2.303 [H^+]_{rbc}}{\beta_{rbc}} \right) \]  \hspace{1cm} (6.6)

\[ V_{rbc}(r) \frac{\partial [HCO_3^-]_{rbc}}{\partial z} = \left( \frac{s}{\nu_{rbc}} \right) \text{Flux}_{\text{HCO}_3} + R_{\text{HCO}_3, rbc} \]  \hspace{1cm} (6.7)

\[ V_{rbc}(r) \frac{\partial [Cl^-]_{rbc}}{\partial z} = -\left( \frac{s}{\nu_{rbc}} \right) \text{Flux}_{\text{HCO}_3} \]  \hspace{1cm} (6.8)

where
\([O_2]_{rbc}\) and \([CO_2]_{rbc}\) are the mean dissolved \(O_2\) and \(CO_2\) concentrations in the RBC, respectively.

\([HbO_2]_{rbc}\) and \([HbCO_2]_{rbc}\) are the concentrations of reduced heme which are in combination with \(O_2\) and \(CO_2\), respectively.

\([O_2HbCO_2]_{rbc}\) is the concentration of oxygenated heme which is in combination with \(CO_2\).

\([H^+]_{rbc}\), \([HCO_3^-]_{rbc}\) and \([Cl^-]_{rbc}\) are the concentrations of \(H^+\), \(HCO_3^-\) and \(Cl^-\) in the RBC, respectively.

\(R_{HbO2}\) is the rate of formation of oxyhemoglobin per unit RBC volume, and
\[ R_{HbO2} = k' [O_2]_{rbc} [Hb]_{rbc} - k [HbO_2]_{rbc}. \]

\(R_{HbCO2}\) is the rate of formation of hemoglobin carbamate per unit RBC volume, and
\[ R_{HbCO2} = k_a [CO_2]_{rbc} [Hb]_{rbc} - \frac{k_d}{K_a} [HbCO_2]_{rbc} [H^+]_{rbc} \] (Forster, 1969).

\(R_{O2HbCO2}\) is the rate of formation of oxyhemoglobin carbamate per unit RBC volume, and
\[ R_{O2HbCO2} = k'_a [CO_2]_{rbc} [HbO_2]_{rbc} - \frac{k'_d}{K_a} [O_2HbCO_2]_{rbc} [H^+]_{rbc} \] (Forster, 1969).

\(R_{HCO3,rbc}\) is the rate of consumption of \(CO_2\) per unit RBC volume by the hydration reaction catalyzed by the intracellular carbonic anhydrase, and
\[ R_{HCO3,rbc} = \hat{A}_{rbc} \left( k_d [CO_2]_{rbc} - \frac{k_v}{K_1 f_{water}} [H^+]_{rbc} [HCO_3^-]_{rbc} \right) \] where \(\hat{A}_{rbc}\) is the intracellular carbonic anhydrase activity factor (Garg and Maren, 1972; Bidani et al., 1978).

\(\sigma_1\) is the amount of \(H^+\) released per \(O_2\) molecule that is bound to hemoglobin (Reaction (6.IV)); literature value of \(\sigma\) is about 0.5-0.7 (Hlastala and Woodson, 1975).

\(\sigma_2\) is the amount of \(H^+\) released per \(CO_2\) molecule that is bound to hemoglobin; literature value of \(\gamma\) is about 1.5-1.8 (Gros et al., 1976). \(\gamma\) is greater than 1 and almost 2 because \(CO_2\) does not react with positively charged amino group, Reaction (6.IV).
In addition, in the physiological range, carbamino hemoglobin exists almost completely in the ionized state, thus Reaction (6.VI).

\[ \sigma_3 \] is the amount of \( H^+ \) released per \( CO_2 \) molecule that is bound to oxyhemoglobin (Reactions (6.IV) and (6.VII)).

\( \text{Flux}_{CO_2} \) is the flux of \( CO_2 \) crossing the RBC wall; it is a function of \( r \) and \( z \) and has the unit of moles per unit area per unit time.

\( \text{Flux}_{HCO_3} \) is the net flux of extracellular \( HCO_3^- \) entering the RBCs; or, equivalently, the net flux of intracellular \( Cl^- \) entering the plasma. It is a function of the intra- and extracellular \( HCO_3^- \) and \( Cl^- \) compositions and is given by Equation (5.11), moles per unit area per unit time.

\( \beta_{rbc} \) is the intracellular hemoglobin buffering factor, equivalents of base per \( pH \) unit.

It should be mentioned that the concentrations for the various species inside the RBC are values averaged within each individual RBC but varying with the position of the RBC in the tube. In addition, these intracellular concentrations are expressed in the basis of per unit RBC volume. Because no hemoglobin escapes from the RBCs, \([Hb]_{total} = [Hb]_{rbc} + [HbO_2]_{rbc} + [HbCO_2]_{rbc} + [O_2HbCO_2]_{rbc}\) remains constant.

Therefore, only three independent mass balances are written for the four different species of hemoglobin that are considered in this study.

**RBC-Rich Plasma Region (0 ≤ r ≤ r_0)**

In the RBC-rich plasma region, the following species are considered.

\[
(1 - h(r)) V_{pl}(r) \frac{\partial [O_2]_{pl}}{\partial z} = \frac{D_{O_2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{pl}}{\partial r} \right) + h(r) \left( \frac{\Sigma}{V_{rbc}} \right) \text{Flux}_{O_2}
\]  
(6.9)
\[
(1 - h(r)) V_{pl}(r) \frac{\partial [CO_2]_{pl}}{\partial z} = \frac{D_{CO2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [CO_2]_{pl}}{\partial r} \right) - h(r) \left( \frac{\partial}{\partial z} \right)_{rbc} \text{Flux}_{CO_2} - (1 - h(r)) R_{HCO3,pl} \tag{6.10}
\]

\[
(1 - h(r)) V_{pl}(r) \frac{\partial [H^+]_{pl}}{\partial z} = \frac{D_{H,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [H^+]_{pl}}{\partial r} \right) + (1 - h(r)) R_{HCO3,pl} \left( \frac{2.303 [H^+]_{pl}}{\beta_{pl}} \right) \tag{6.11}
\]

\[
(1 - h(r)) V_{pl}(r) \frac{\partial [HCO_3^-]_{pl}}{\partial z} = \frac{D_{HCO3,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [HCO_3^-]_{pl}}{\partial r} \right) - h(r) \left( \frac{\partial}{\partial z} \right)_{rbc} \text{Flux}_{HCO3} + (1 - h(r)) R_{HCO3,pl} \tag{6.12}
\]

\[
(1 - h(r)) V_{pl}(r) \frac{\partial [Cl^-]_{pl}}{\partial z} = \frac{D_{Cl,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [Cl^-]_{pl}}{\partial r} \right) + h(r) \left( \frac{\partial}{\partial z} \right)_{rbc} \text{Flux}_{HCO3} \tag{6.13}
\]

where

\([O_2]_{pl}\) and \([CO_2]_{pl}\) are the dissolved \(O_2\) and \(CO_2\) concentrations, respectively, in the RBC-rich plasma.

\([H^+]_{pl}, [HCO_3^-]_{pl}\) and \([Cl^-]_{pl}\) are the concentrations of \(H^+\), \(HCO_3^-\) and \(Cl^-\) in the RBC-rich plasma, respectively.

\(R_{HCO3,pl}\) is the rate of consumption of \(CO_2\) per unit plasma volume by the hydration reaction, and \(R_{HCO3,pl} = \hat{A}_{pl} \left( k_u [CO_2]_{pl} - \frac{k_v}{K_1} [H^+]_{pl} [HCO_3^-]_{pl} \right)\) where \(\hat{A}_{pl}\) is the extracellular carbonic anhydrase activity factor.

\(D_{O2,pl}\) and \(D_{CO2,pl}\) are the molecular diffusivities of \(O_2\) and \(CO_2\) in the plasma, respectively.

\(D_{H,pl}, D_{HCO3,pl}\) and \(D_{Cl,pl}\) are the effective ionic diffusivities of \(H^+\), \(HCO_3^-\) and \(Cl^-\) in the plasma, respectively.

\(\beta_{pl}\) is the extracellular plasma buffering capacity, equivalents of base per \(pH\) unit.
In the RBC-free plasma region, the continuity equations for various species can be obtained from the mass balances for various species in the RBC-rich plasma region by requiring \( h(r) \) to be zero.

\[
V'_{pl}(r) \frac{\partial [O_2]_{pl}'}{\partial z} = \frac{D_{O_2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{pl}'}{\partial r} \right)
\]

(6.14)

\[
V'_{pl}(r) \frac{\partial [CO_2]_{pl}'}{\partial z} = \frac{D_{CO_2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [CO_2]_{pl}'}{\partial r} \right) - R'_{HCO3,pl}
\]

(6.15)

\[
V'_{pl}(r) \frac{\partial [H^+]_{pl}'}{\partial z} = \frac{D_{H,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [H^+]_{pl}'}{\partial r} \right) + R'_{HCO3,pl} \left( \frac{2.303 \cdot [H^+]_{pl}'}{\beta_{pl}} \right)
\]

(6.16)

\[
V'_{pl}(r) \frac{\partial [HCO_3^-]_{pl}'}{\partial z} = \frac{D_{HCO3,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [HCO_3^-]_{pl}'}{\partial r} \right) + R'_{HCO3,pl}
\]

(6.17)

\[
V'_{pl}(r) \frac{\partial [Cl^-]_{pl}'}{\partial z} = \frac{D_{Cl,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [Cl^-]_{pl}'}{\partial r} \right)
\]

(6.18)

where

\([O_2]_{pl}'\) and \([CO_2]_{pl}'\) are the dissolved \( O_2 \) and \( CO_2 \) concentrations in the RBC-free plasma, respectively.

\([H^+]_{pl}', [HCO_3^-]_{pl}'\) and \([Cl^-]_{pl}'\) are the concentrations of \( H^+ \), \( HCO_3^- \) and \( Cl^- \) in the RBC-free plasma, respectively.

\( R'_{HCO3,pl} \) is the rate of consumption of \( CO_2 \) per unit plasma volume by the hydration reaction, and \( R'_{HCO3,pl} = \hat{A}_{pl} (k_u [CO_2]_{pl}' - \frac{k_v}{K_1} [H^+]_{pl}' [HCO_3^-]_{pl}') \).
6.1.3 Simplification of Transport Equations

6.1.3.a Type I Model (10 PDEs)

To understand the basic aspects of gas transport and pH regulation by flowing blood, simplifications are made to render the problem tractable from a mathematical point of view, while still retaining the terms in the continuity equations which are believed to be primarily important. Basically the following assumptions are introduced to simplify the above model which consists a system of 18 simultaneous nonlinear PDEs.

From the detailed O₂ transport studies, it was reported that only a small fraction of the resistance to transport lies inside the RBCs. The carbonic anhydrase enzyme which is present in high concentration in RBCs speeds up the CO₂ hydration/dehydration reactions by approximately 6,500 fold. In addition, because CO₂ has higher solubilities in the blood than O₂ (see Tables 6.3 and 6.4) and that the diffusion of CO₂ across the RBC membrane has been shown to be very rapid (Gros and Moll, 1971), it is assumed that the diffusion occurs rapidly enough that any $P_{CO₂}$ gradient across the RBC membrane is negligible. Therefore, assumptions of chemical equilibrium within the RBC and negligible intra- and extracellular boundary resistances are appropriate for describing the transport of both O₂ and CO₂.

By combining Equations (6.1), (6.2) and (6.5) and invoking the chemical equilibrium, one obtains

$$V_{\text{rbc}}(r) \left( 1 + \frac{\partial [HbO₂]_{\text{rbc}}}{\partial [O₂]_{\text{rbc}}} + \frac{\partial [O₂HbCO₂]_{\text{rbc}}}{\partial [O₂]_{\text{rbc}}} \right) \frac{\partial [O₂]_{\text{rbc}}}{\partial z} = - \left( \frac{L}{V} \right)_{\text{rbc}} \text{Flux}_{O₂} \tag{6.19}$$

Because chemical equilibrium inside the RBC is imposed, oxyhemoglobin and oxyhemoglobin carbamate can be incorporated into the dissolved O₂ equation. If the Hill model, Equation (4.16a), is chosen to describe the ODC, then the slope of the
ODC is specified by Equation (4.16b) where \( P_{50} \) is a function of \( P_{CO2}, pH, [DPG] \) and is given by Equation (2.2). On the other hand, if the modified Easton model, Equation (2.5), is selected to describe the dissociation curve, then \( \partial[HbO_2]_{rbc}/\partial[O_2]_{rbc} \) is given by

\[
\frac{\partial[HbO_2]_{rbc}}{\partial[O_2]_{rbc}} = \frac{C_{heme,rbc} \kappa}{\alpha O_2,rbc} (S_{max} - S_{min}) \exp[\kappa(P_{O2}^* - P_{O2})] \exp[- \exp[\kappa(P_{O2}^* - P_{O2})]]
\]

(6.20)

where \( \kappa \) is a function of \( P_{CO2}, pH, [DPG] \) and is characterized by Equation (2.5).

For an approximation, it is also assumed that \( \partial[O_2HbCO_2]_{rbc}/\partial[O_2]_{rbc} \ll (1+\partial[HbO_2]_{rbc}/\partial[O_2]_{rbc}) \). The reason is that at every \( pH \) value reduced hemoglobin has a much greater ability to bind \( CO_2 \) than does oxyhemoglobin, and this difference increases with \( pH \); thus Reaction (6.7) is of secondary importance in comparison to Reaction (6.6). Moreover, available literature data is insufficient for the estimation of this derivative.

In a similar fashion, Equations (6.3), (6.4), (6.5) and (6.7) are combined to give Equation (6.21). Again, because the chemical equilibrium assumption is enforced intracellularly, both carbamino hemoglobin species and \( HCO_3^- \) can be incorporated into the dissolved \( CO_2 \) equation.

\[
V_{rbc}(r) \left( 1 + \frac{\partial[HbCO_2]_{total}}{\partial[CO_2]_{rbc}} + \frac{\partial[HCO_3^-]_{rbc}}{\partial[CO_2]_{rbc}} \right) \frac{\partial[CO_2]_{rbc}}{\partial z} = \left( \frac{S}{V} \right)_{rbc} Flux_{CO2} + \left( \frac{S}{V} \right)_{rbc} Flux_{HCO3}
\]

(6.21)

where \([HbCO_2]_{total} = [HbCO_2]_{rbc} + [O_2HbCO_2]_{rbc}\). If Equation (2.10) is chosen to describe the total carbamino \( CO_2 \) bound by the hemoglobin, this derivative, \( \partial[HbCO_2]_{total}/\partial[CO_2]_{rbc} \), is given by
\[
\frac{\partial (HbCO_2)_{\text{total}}}{\partial [CO_2]_{\text{rcb}}} = \frac{C_{\text{phys.}}}{4} \left[ \frac{2\lambda_\alpha + 4\lambda_\alpha^2 [CO_2]_{\text{rcb}}}{(1 + \lambda_\alpha [CO_2]_{\text{rcb}})^2} + \frac{2\lambda_\beta (1 + K_{DPG}[DPG]) + 4\lambda_\beta^2 [CO_2]_{\text{rcb}}}{(1 + \lambda_\beta [CO_2]_{\text{rcb}} + K_{DPG}[DPG])^2} \right]
\]
(6.22)

If the Henderson-Hasselbalch equation (Equation (2.9a)) is used to quantify the equilibrium relationship between the interacting species that are involved in the CO₂ hydration/dehydration reactions, then \( \partial [HCO_3^-]_{\text{rcb}}/\partial [CO_2]_{\text{rcb}} = K' f_{\text{water}}/[H^+]_{\text{rcb}} \).

Another assumption introduced here is that the reaction term \( R_{O2HbCO2} \) is neglected in the derivation of Equation (6.23) for the reason mentioned previously that Reaction (6.VII) is of less importance than Reaction (6.VI) under physiologically relevant conditions. Multiplication of Equation (6.2) by \( -\sigma_1 \), Equation (6.4) by \( -\sigma_2 \); Equation (6.7) by \( -1 \), Equation (6.6) by \( \beta_{\text{rcb}}/(2.303[H^+]_{\text{rcb}}) \), and addition of the above resultant equations yield the following equation:

\[
V_{\text{rcb}}(r) \left( \frac{\beta_{\text{rcb}}}{2.303 \ [H^+]_{\text{rcb}}} - \sigma_1 \frac{\partial [HbO_2]_{\text{rcb}}}{\partial [H^+]_{\text{rcb}}} - \sigma_2 \frac{\partial [HbCO_2]_{\text{rcb}}}{\partial [H^+]_{\text{rcb}}} - \frac{\partial [HCO_3^-]_{\text{rcb}}}{\partial [H^+]_{\text{rcb}}} \right) \frac{\partial [H^+]_{\text{rcb}}}{\partial z} = -\left( \frac{\delta}{V_{\text{rcb}}} \right) \text{Flux}_{HCO_3^-}
\]
(6.23)

where

\( \partial [HbO_2]_{\text{rcb}} / \partial [H^+]_{\text{rcb}} \) is related to the inverse of the number of release or gain of protons by hemoglobin protein accompanying oxygenation or deoxygenation; i.e.,
\( \partial [HbO_2]_{\text{rcb}} / \partial [H^+]_{\text{rcb}} = \sigma_1^{-1} \).

\( \partial [HbCO_2]_{\text{rcb}} / \partial [H^+]_{\text{rcb}} \) is related to the inverse of the number of gain or lose of protons by hemoglobin protein accompanying release or uptake of CO₂; i.e.,
\( \partial [HbCO_2]_{\text{rcb}} / \partial [H^+]_{\text{rcb}} = \sigma_2^{-1} \).

\( \partial [HCO_3^-]_{\text{rcb}} / \partial [H^+]_{\text{rcb}} = -\left( K' f_{\text{water}} [CO_2]_{\text{rcb}} \right)/[H^+]_{\text{rcb}}^2 \) which is obtained through Equation (2.9a).
It is also assumed that there is no transport resistance in the RBC membrane for O\(_2\) and CO\(_2\); thus \(P_{O2}\) and \(P_{CO2}\) are continuous at the RBC wall. In addition, if \(\alpha_{O2, rbc} = \alpha_{O2, pl}\) and \(\alpha_{CO2, rbc} = \alpha_{CO2, pl}\), then \([O_2]_{r,w, rbc} = [O_2]_{r,w, pl}\) and \([CO_2]_{r,w, rbc} = [CO_2]_{r,w, pl}\) where \([O_2]_{r,w, rbc}\) and \([CO_2]_{r,w, rbc}\) are the concentrations of \(O_2\) and \(CO_2\), respectively, at the RBC wall in the RBC; and \([O_2]_{r,w, pl}\) and \([CO_2]_{r,w, pl}\) are the concentrations of \(O_2\) and \(CO_2\), respectively, at the RBC wall in the plasma. Furthermore, if the intra- and extracellular boundary resistances are neglected (i.e., \([O_2]_{rbc} = [O_2]_{r,w, rbc}\), \([O_2]_{pl} = [O_2]_{r,w, pl}\) and \([CO_2]_{rbc} = [CO_2]_{r,w, rbc}\), \([CO_2]_{pl} = [CO_2]_{r,w, pl}\)) then it follows that

\[
[O_2]_{pl} = \left(\frac{\alpha_{O2, pl}}{\alpha_{O2, rbc}}\right) [O_2]_{rbc}
\]

(6.24)

\[
[CO_2]_{pl} = \left(\frac{\alpha_{CO2, pl}}{\alpha_{CO2, rbc}}\right) [CO_2]_{rbc}
\]

(6.25)

Multiplication of Equation (6.19) by \(h(r)\), addition of the resultant expression to Equation (6.9) and substitution of \((\alpha_{O2, pl} / \alpha_{O2, rbc})[O_2]_{rbc}\) for \([O_2]_{pl}\) yields the following mass balance for dissolved \(O_2\) in the RBC:

\[
\left[\left(\frac{\alpha_{O2, rbc}}{\alpha_{O2, pl}}\right) h(r) V_{rbc}(r)\left(1 + \frac{\partial[HbO_2]_{rbc}}{\partial [O_2]_{rbc}}\right) + (1 - h(r)) V_{pl}(r)\right] \frac{\partial [O_2]_{rbc}}{\partial z} =
\]

\[
\frac{D_{O2, pl}}{r} \frac{\partial}{\partial r} \left(r \frac{\partial [O_2]_{rbc}}{\partial r}\right)
\]

(6.26)

In an analogous fashion, Equations (6.21) and (6.10) can be combined to give the following mass balance for dissolved \(CO_2\):
\[
\left[ h(r) V_{rbc}(r) \left( 1 + \frac{\partial[HCO_3^-]_{rbc}}{\partial[CO_2]_{rbc}} + \frac{\partial[HbCO_2]_{\text{total}}}{\partial[CO_2]_{rbc}} \right) + \frac{\alpha_{CO2,pl}}{\alpha_{CO2,rbc}} (1 - h(r)) V_{pl}(r) \right] \frac{\partial[CO_2]_{rbc}}{\partial z}
\]

\[
= \frac{\alpha_{CO2,pl}}{\alpha_{CO2,rbc}} \frac{D_{CO2,pl}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[CO_2]_{rbc}}{\partial r} \right) + h(r) \left( \frac{\delta}{V_{rbc}} \right) \text{Flux}_{HCO_3} - (1 - h(r)) R_{HCO_3,pl}
\]

(6.27)

To further simplify the model, the mass balances for dissolved O\(_2\) and Cl\(^-\) in the cell-free plasma region, Equations (6.14) and (6.18), can be solved analytically and incorporated as boundary conditions to Equations (6.26) and (6.13), respectively. This approach uses the finding that convective transport of these species in the cell-free plasma region is small relative to transport by diffusion. Hence, the term \(V_{pl} \partial[C_i]/\partial z\), where \(i = O_2\) or Cl\(^-\), can be dropped from Equations (6.14) and (6.18). The simplified equations were then solved subject to conditions of a specified concentration at \(r=r_r\), the RBC-rich and RBC-free region interface; and continuity of flux at \(r=r_c\), the vessel wall. The solutions to these simplified equations can then be incorporated as boundary conditions to Equations (6.26) and (6.13), respectively, at \(r=r_r\).

After introducing the above assumptions, the original model is simplified down to a model with 10 PDEs which is summarized in Table 6.1:
Table 6.1: Type I Model. Partial differential equations describing the coupled O$_2$ and CO$_2$ transport and pH regulation by blood flowing in microvessels.

\[
\left[ \frac{\alpha_{O_2,\text{bc}}}{\alpha_{O_2,\text{pl}}} \right] h(r) V_{\text{bc}}(r) \left( 1 + \frac{\partial[HbO_2]_{\text{bc}}}{\partial[O_2]_{\text{bc}}} \right) + (1 - h(r)) V_{p}(r) \frac{\partial[O_2]_{\text{bc}}}{\partial z} = \frac{D_{O_2,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[O_2]_{\text{bc}}}{\partial r} \right)
\]  

(6.1.1)

\[
\left[ h(r) V_{\text{bc}}(r) \right] \left( 1 + \frac{\partial[HCO_3^-]_{\text{bc}}}{\partial[CO_2]_{\text{bc}}} + \frac{\partial[HbCO_2]_{\text{total}}}{\partial[CO_2]_{\text{bc}}} \right) + \frac{\alpha_{CO_2,\text{pl}}}{\alpha_{CO_2,\text{bc}}} (1 - h(r)) V_{p}(r) \frac{\partial[CO_2]_{\text{bc}}}{\partial z} = \frac{D_{CO_2,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[CO_2]_{\text{bc}}}{\partial r} \right) + h(r) \left( \frac{\partial}{\partial r} \right) \text{Flux}_{\text{HCO}_3} \cdot (1 - h(r)) R_{\text{HCO}_3,\text{pl}}
\]

(6.1.2)

\[
V_{\text{bc}}(r) \frac{\partial H^+_{\text{bc}}}{\partial z} = - \left( \frac{\partial}{\partial r} \right) \text{Flux}_{\text{HCO}_3}
\]

(6.1.3)

\[
(1 - h(r)) V_{p}(r) \frac{\partial H^+_{\text{pl}}}{\partial z} = \frac{D_{H^+,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial H^+_{\text{pl}}}{\partial r} \right) + (1 - h(r)) R_{\text{HCO}_3,\text{pl}} \left( \frac{2.303 \ [H^+]_{\text{pl}}}{\beta_{\text{H}}^+} \right)
\]

(6.1.5)

\[
(1 - h(r)) V_{p}(r) \frac{\partial[HCO_3^-]_{\text{pl}}}{\partial z} = \frac{D_{HCO_3,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[HCO_3^-]_{\text{pl}}}{\partial r} \right) \cdot h(r) \left( \frac{\partial}{\partial r} \right) \text{Flux}_{\text{HCO}_3} + (1 - h(r)) R_{\text{HCO}_3,\text{pl}}
\]

(6.1.6)

\[
(1 - h(r)) V_{p}(r) \frac{\partial[Cl^-]_{\text{pl}}}{\partial z} = \frac{D_{Cl^-,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[Cl^-]_{\text{pl}}}{\partial r} \right) + h(r) \left( \frac{\partial}{\partial r} \right) \text{Flux}_{\text{HCO}_3}
\]

(6.1.7)

\[
V_{p}(r) \frac{\partial[CO_2]_{\text{pl}}}{\partial z} = \frac{D_{CO_2,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[CO_2]_{\text{pl}}}{\partial r} \right) - R_{\text{HCO}_3,\text{pl}}
\]

(6.1.8)

\[
V_{p}(r) \frac{\partial[H^+]_{\text{pl}}}{\partial z} = \frac{D_{H^+,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[H^+]_{\text{pl}}}{\partial r} \right) + R_{\text{HCO}_3,\text{pl}} \left( \frac{2.303 \ [H^+]_{\text{pl}}}{\beta_{\text{H}}^+} \right)
\]

(6.1.9)

\[
V_{p}(r) \frac{\partial[HCO_3^-]_{\text{pl}}}{\partial z} = \frac{D_{HCO_3,\text{pl}}}{r} \frac{\partial}{\partial r} \left( r \frac{\partial[HCO_3^-]_{\text{pl}}}{\partial r} \right) + R_{\text{HCO}_3,\text{pl}}
\]

(6.1.10)
Finally, to complete the mathematical description, inlet and boundary conditions are imposed. At lines of symmetry, the following boundary conditions hold:

\[
\frac{\partial [O_2]_{bc}}{\partial r} = 0
\]  
(6.28a)

\[
\frac{\partial [CO_2]_{bc}}{\partial r} = 0
\]  
(6.28b)

\[
\frac{\partial [H^+]_{pl}}{\partial r} = 0
\]  
(6.28c)

\[
\frac{\partial [HCO_3^-]_{pl}}{\partial r} = 0
\]  
(6.28d)

\[
\frac{\partial [Cl^-]_{pl}}{\partial r} = 0
\]  
(6.28e)

At the RBC-rich and RBC-free region interface, the modified flux boundary conditions for \(O_2\) and \(Cl^-\) and continuity of concentration and flux for the other species are imposed:

\[
-D_{O_2,pl} \frac{\partial [O_2]_{bc}}{\partial r} = -D_{O_2,pl} \left( \alpha_{O_2,rc} P_{O_2,ext} - [O_2]_{bc} \right)
\]

\[
\frac{D_{O_2,pl}}{r_r} \left( \frac{\alpha_{O_2,pl} \ln(GF)}{K_{O_2}} + \ln \left( \frac{r_c}{r_r} \right) \right)
\]  
(6.29a)

\[
\left( \frac{\alpha_{CO_2,pl}}{\alpha_{CO_2,rc}} \right) [CO_2]_{bc} = [CO_2]_{pl}^i
\]  
(6.29b)

\[
-D_{CO_2,pl} \left( \frac{\alpha_{CO_2,pl}}{\alpha_{CO_2,rc}} \right) \frac{\partial [CO_2]_{bc}}{\partial r} = -D_{CO_2,pl} \frac{\partial [CO_2]_{pl}^i}{\partial r}
\]  
(6.29c)

\[
[H^+]_{pl} = [H^+]_{pl}^i
\]  
(6.29d)
\[- \tilde{D}_{H,pl} \frac{\partial [H^+]_{pl}}{\partial r} = - \tilde{D}_{H,pl} \frac{\partial [H^+]_{pl}}{\partial r} \]
\[= - \tilde{D}_{H,pl} \frac{\partial [H^+]_{pl}}{\partial r} \]
\[
(6.29e)
\]
\[[HCO_3^-]_{pl} = [HCO_3^-]_{pl}'
\[
(6.29f)
\]
\[- \tilde{D}_{HCO_3,pl} \frac{\partial [HCO_3^-]_{pl}}{\partial r} = - \tilde{D}_{HCO_3,pl} \frac{\partial [HCO_3^-]_{pl}}{\partial r} \]
\[
(6.29g)
\]
\[- \tilde{D}_{Cl,pl} \frac{\partial [Cl^-]_{pl}}{\partial r} = 0 \]
\[
(6.29h)
\]

At the vessel wall, the following flux conditions are prescribed:

\[- D_{CO_2,pl} \frac{\partial [CO_2]_{pl}'}{\partial r} = \frac{K_{CO_2}}{\alpha_{CO_2,pl} r_e \ln(GF)} ([CO_2]_{pl}' - \alpha_{CO_2,pl} P_{CO_2,ext}) \]
\[
(6.30a)
\]
\[- \tilde{D}_{H,pl} \frac{\partial [H^+]_{pl}'}{\partial r} = 0 \]
\[
(6.30b)
\]
\[- \tilde{D}_{HCO_3,pl} \frac{\partial [HCO_3^-]_{pl}'}{\partial r} = 0 \]
\[
(6.30c)
\]

where

\( K_{O_2} \) and \( K_{CO_2} \) are the gas permeability constants of the artificial membrane film for \( O_2 \) and \( CO_2 \), respectively.

\( P_{O_2,ext} \) and \( P_{CO_2,ext} \) are the partial pressures of \( O_2 \) and \( CO_2 \), respectively, in the gas space surrounding the artificial membrane tubes.

At the entrance, the compositions are assumed to be uniform and in equilibrium.

\([O_2]_{rbc} = [O_2]_n \]
\[
(6.31a)
\]
\[ [CO_2]_{rbc} = [CO_2]_{in} \quad (6.31b) \]
\[ [H^+]_{rbc} = [H^+]_{rbc, in} \quad (6.31c) \]
\[ [Cl^-]_{rbc} = R_{GD} [Cl^-]_{pl, in} \quad (6.31d) \]
\[ [H^+]_{pl} = [H^+]_{pl, in} \quad (6.31e) \]
\[ [HCO_3^-]_{pl} = \frac{K' \left( \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \right) [CO_2]_{rbc, in}}{[H^+]_{pl, in}} \quad (6.31f) \]
\[ [Cl^-]_{pl} = [Cl^-]_{pl, in} \quad (6.31g) \]
\[ [CO_2]_{pl} = \left( \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \right) [CO_2]_{in} \quad (6.31h) \]
\[ [H^+]_{pl} = [H^+]_{pl, in} \quad (6.31i) \]
\[ [HCO_3^-]_{pl} = \frac{K' \left( \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \right) [CO_2]_{rbc, in}}{[H^+]_{pl, in}} \quad (6.31j) \]

where \( R_{GD} = \left( \frac{[HCO_3^-]_{rbc, in}}{[HCO_3^-]_{pl, in}} \right) = K' f_{water} [CO_2]_{rbc, in} \frac{[H^+]_{rbc, in}}{[HCO_3^-]_{pl, in}}. \)

### 6.1.3.2 Type II Model (7 PDEs)

This transport model can be further reduced to a system of 7 simultaneous, nonlinear PDEs consisting of Equations (6.1.1) - (6.1.7) by incorporating the continuity equations for \( CO_2 \), \( H^+ \) and \( HCO_3^- \) in the RBC-free plasma region, Equations (6.1.8) - (6.1.10), as boundary conditions to Equations (6.1.2), (6.1.5) and (6.1.6), respectively. Again, these solutions use the finding that convective transport of various species is small relative to transport by diffusion. Hence the convective transport terms can be dropped from Equations (6.1.8) - (6.1.10). Additionally, the reaction term, \( R_{HCO_3, pl} \) in these equations is deleted for the reason that the \( CO_2 \)
hydration/dehydration reaction is very slow in the absence of the carbonic anhydrase enzyme. Even if there were some carbonic anhydrase enzyme added to the extracellular medium to speed up the reaction, it will still be reasonable to neglect $R^\prime_{HCO_3,pl}$ because the RBC-free region represents only a small fraction of the vessel. Therefore, the simplified RBC-free plasma phase mass balances for $CO_2$, $H^+$ and $HCO_3^-$ are solved analytically and incorporated as boundary conditions at $r=r_r$:

$$- D_{CO_2,pl} \frac{\partial [CO_2]_{rbc}}{\partial r} = - D_{CO_2,pl} \left( \alpha_{CO_2,rbc} P_{CO_2,ext} - [CO_2]_{rbc} \right) \frac{r}{r_r} \left[ \frac{D_{CO_2,pl} \alpha_{CO_2,pl} \ln(GF)}{K_{CO_2}} + \ln \left( \frac{r_e}{r_r} \right) \right]$$  \hspace{1cm} (6.32a)

$$- D_{H^+,pl} \frac{\partial [H^+]_{pl}}{\partial r} = 0 \hspace{1cm} (6.32b)$$

$$- D_{HCO_3,pl} \frac{\partial [HCO_3^-]_{pl}}{\partial r} = 0 \hspace{1cm} (6.32c)$$

### 6.1.4 Dimensionless Equations and Conditions

Before solving these equations, it will be convenient to rewrite them in terms of the following dimensionless variables:

$$t = \frac{z}{L}, \hspace{0.5cm} x = \frac{r}{r_r}, \hspace{0.5cm} x' = \frac{r_e - r}{r_e - r_r},$$

$$u = \frac{[O_2]_{rbc}}{[O_2]_o}, \hspace{0.5cm} v = \frac{[CO_2]_{rbc}}{[CO_2]_o}, \hspace{0.5cm} w = \frac{[H^+]_{rbc}}{[H^+]_o}, \hspace{0.5cm} s = \frac{[Cl^-]_{rbc}}{[Cl^-]_o},$$

$$w' = \frac{[H^+]_{pl}}{[H^+]_o}, \hspace{0.5cm} r' = \frac{[HCO_3^-]_{pl}}{[HCO_3^-]_o}, \hspace{0.5cm} s' = \frac{[Cl^-]_{pl}}{[Cl^-]_o},$$

$$v'' = \frac{[CO_2]_{pl}}{[CO_2]_o}, \hspace{0.5cm} w'' = \frac{[H^+]_{pl}}{[H^+]_o}, \hspace{0.5cm} r'' = \frac{[HCO_3^-]_{pl}}{[HCO_3^-]_o} \hspace{1cm} (6.33)$$

where
$L$ is the length of the artificial membrane tube.

$[O_2]_o, [CO_2]_o, [H^+]_o, [HCO_3^-]_o$ and $[Cl^-]_o$ are the normalized concentrations for various species.

$t$ is the dimensionless axial coordinate.

$x$ and $x'$ are the dimensionless radial coordinates in the RBC-rich and RBC-free plasma regions, respectively.

$u, v, w$ and $s$ are the dimensionless concentrations of $O_2, CO_2, H^+$ and $Cl^-$ inside the RBCs.

$w', r'$ and $s'$ are the dimensionless concentrations of $H^+, HCO_3^-,$ and $Cl^-$ in the RBC-rich plasma region.

$\nu', w''$ and $r''$ are the dimensionless concentrations of $CO_2, H^+, \text{ and } HCO_3^-$ in the RBC-free plasma region.

Details on the dedimensionalization of Type I model are outlined in Appendix C. In terms of the dimensionless variables, the partial differential equations and boundary and inlet conditions may be written

\[
\frac{\partial u}{\partial t} = \frac{a_{2u} \frac{1}{x} \frac{\partial}{\partial x} \left[ x \frac{\partial u}{\partial x} \right]}{\left[ \frac{1}{a_{1u}} h(x) (1-slp)(1-Bb^2x^2) \left( 1 + a_{6u} \frac{\partial s}{\partial t} \right) + (1-h(x)) \left( 1-Bb^2x^2 \right) \right]} \tag{6.34}
\]

\[
\frac{\partial v}{\partial t} = \frac{a_{1v} a_{2v} \frac{1}{x} \frac{\partial}{\partial x} \left[ x \frac{\partial v}{\partial x} \right] + a_{7} a_{4v} \left( \frac{1}{d_{4}} \right) Flux_{HCO_3}^* \left( 1-h(x) \right) R_{HCO_3,pl}^*}{\left[ h(x) (1-slp)(1-Bb^2x^2) \left( 1 + a_{6v} \frac{\partial \phi}{\partial v} + a_{6w} \frac{1}{w} \right) + a_{1v} (1-h(x)) \left( 1-Bb^2x^2 \right) \right]} \tag{6.35}
\]
\[ \frac{\partial_w}{\partial t} = \frac{-\alpha_1 a_{4b} \left( \frac{d_3}{d_4} \right) \text{Flux}_{HCO_3}^*}{(1-slp)(1-Bb^2x^2)} \left[ \frac{1}{d_{5w}} \left( \frac{1}{w} + a_{12w} d_3 \frac{v}{w^2} - 2 \right) \right] \]  

(6.36)

\[ \frac{\partial s}{\partial t} = -\alpha_1 a_{4b} \text{Flux}_{HCO_3}^* \frac{(1-slp)(1-Bb^2x^2)}{(1-Bb^2x^2)} \]  

(6.37)

\[ \frac{\partial w'}{\partial t} = \frac{a_{2w} \left( \frac{1}{x} \frac{\partial}{\partial x} \left( x \frac{\partial w'}{\partial x} \right) + a_5 \frac{d_3}{d_{5w}} (1-h(x)) w' R_{HCO_3,pl}^* \right)}{(1-h(x))(1-Bb^2x^2)} \]  

(6.38)

\[ \frac{\partial r'}{\partial t} = \frac{a_{2r} \left( \frac{1}{x} \frac{\partial}{\partial x} \left( x \frac{\partial r'}{\partial x} \right) - \alpha_1 a_{4b} h(x) \text{Flux}_{HCO_3}^* + d_4 (1-h(x)) R_{HCO_3,pl}^* \right)}{(1-h(x))(1-Bb^2x^2)} \]  

(6.39)

\[ \frac{\partial s'}{\partial t} = \frac{a_{2s} \left( \frac{1}{x} \frac{\partial}{\partial x} \left( x \frac{\partial s'}{\partial x} \right) + \alpha_1 a_{4b} d_5 h(x) \text{Flux}_{HCO_3}^* \right)}{(1-h(x))(1-Bb^2x^2)} \]  

(6.40)

\[ \frac{\partial v''}{\partial t} = \frac{\alpha_{8v1} \left( \frac{1}{x^2} \frac{\partial^2 v''}{\partial x'^2} - \alpha_{8v2} \left[ \frac{1}{1-(1-b)x'} \right] \frac{\partial v''}{\partial x'} \right) - \left( \frac{1}{1-b} \right) R_{HCO_3,pl}^*}{[2 - (1-b)x'] x'} \]  

(6.41)

\[ \frac{\partial w''}{\partial t} = \frac{\alpha_{8w1} \left( \frac{1}{x^2} \frac{\partial^2 w''}{\partial x'^2} - \alpha_{8w2} \left[ \frac{1}{1-(1-b)x'} \right] \frac{\partial w''}{\partial x'} \right) + a_5 d_3 \left( \frac{1}{1-b} \right) w'' R_{HCO_3,pl}^*}{[2 - (1-b)x'] x'} \]  

(6.42)

\[ \frac{\partial r''}{\partial t} = \frac{\alpha_{8r1} \left( \frac{1}{x^2} \frac{\partial^2 r''}{\partial x'^2} - \alpha_{8r2} \left[ \frac{1}{1-(1-b)x'} \right] \frac{\partial r''}{\partial x'} \right) + d_4 \left( \frac{1}{1-b} \right) R_{HCO_3,pl}^*}{[2 - (1-b)x'] x'} \]  

(6.43)
where

\[
\text{Flux}^*_\text{HCO}_3 = \frac{d_1 a_{4u} (s' r' - s' r)}{(s + s') + d_5 (r + r') + 2 \left[ d_6 s s' + d_7 r' r + d_8 (s' r' + s' r') \right]}. 
\]

\[
\hat{R}^*_{\text{HCO}_3, pl} = \hat{A}_{pl} (a_{4ku} a_{1u} v - a_{4kv} d_1 w' r') . 
\]

\[
\hat{R}^*_{\text{HCO}_3, pl} = \hat{A}_{pl} (a_{3ku} v'' - a_{3kv} d_1 w'' r'') . 
\]

The boundary conditions that go with the simplified equations for type I model
are listed below. At lines of symmetry,

\[
\frac{\partial u}{\partial x} = 0 \quad (6.44a) 
\]

\[
\frac{\partial v}{\partial x} = 0 \quad (6.44b) 
\]

\[
\frac{\partial w'}{\partial x} = 0 \quad (6.44c) 
\]

\[
\frac{\partial r'}{\partial x} = 0 \quad (6.44d) 
\]

\[
\frac{\partial s'}{\partial x} = 0 \quad (6.44e) 
\]

At the RBC-rich and RBC-free region interface,

\[
\frac{\partial u}{\partial x} + a_{3u}(u - u_{\text{ext}}) = 0 \quad (6.45a) 
\]

\[
a_{1v} v - v'' = 0 \quad (6.45b) 
\]

\[
a_{1v} \frac{\partial v}{\partial x} + \left( \frac{b}{1 - b} \right) \frac{\partial v''}{\partial x'} = 0 \quad (6.45c) 
\]

\[
w' - w'' = 0 \quad (6.45d) 
\]
\[
\frac{\partial w'}{\partial x} + \left(\frac{b}{1 - b}\right) \frac{\partial w''}{\partial x'} = 0
\]  
(6.45e)

\[
 r' - r'' = 0
\]  
(6.45f)

\[
\frac{\partial r'}{\partial x} + \left(\frac{b}{1 - b}\right) \frac{\partial r''}{\partial x'} = 0
\]  
(6.45g)

\[
\frac{\partial s'}{\partial x} = 0
\]  
(6.45h)

At the vessel wall,

\[
\frac{\partial v''}{\partial x'} - a_3 v'' (v'' - a_1 v v_{ext}) = 0
\]  
(6.46a)

\[
\frac{\partial w''}{\partial x'} = 0
\]  
(6.46b)

\[
\frac{\partial r''}{\partial x'} = 0
\]  
(6.46c)

At the entrance,

\[
u = u_{in}
\]  
(6.47a)

\[
v = v_{in}
\]  
(6.47b)

\[
w = w_{rbc, in}
\]  
(6.47c)

\[
s = R_{GD} s_{pl, in}
\]  
(6.47d)

\[
w' = w_{pl, in}
\]  
(6.47e)

\[
r' = \left(\frac{a_1 v d_2}{d_1}\right) \frac{v_{in}}{w_{pl, in}}
\]  
(6.47f)

\[
s' = s_{pl, in}
\]  
(6.47g)

\[
v'' = v_{in}
\]  
(6.47h)

\[
w'' = w_{pl, in}
\]  
(6.47i)
\[ r^* = \left( \frac{a_{1u} d_9}{d_1} \right) \frac{v_{in}}{w_{pl,in}} \]

(6.47j)

where

\[ a_{1u} = \frac{\alpha_{O_{2,pl}}}{\alpha_{O_{2,rbc}}}, \quad a_{1v} = \frac{\alpha_{CO_{2,pl}}}{\alpha_{CO_{2,rbc}}} \]

\[ a_{2u} = \frac{D_{O_{2,pl}} L}{D r_f^2}, \quad a_{2v} = \frac{D_{CO_{2,pl}} L}{D r_f^2}, \quad a_{2w} = \frac{D_{Hpl} L}{D r_f^2}, \quad a_{2r} = \frac{D_{HCO_{3,pl}} L}{D r_f^2}, \quad a_{2s} = \frac{D_{Cl,pl} L}{D r_f^2}, \]

\[ a_{3u} = \frac{K_{O_{2}}}{D_{O_{2,pl}} \alpha_{O_{2,pl}} \ln(GF) + K_{O_{2}} \ln\left(\frac{r_e}{r_r}\right)}, \quad a_{3v} = \frac{K_{CO_{2}} \left(1 - \frac{r_f}{r_e}\right)}{D_{CO_{2,pl}} \alpha_{CO_{2,pl}} \ln(GF)}, \]

\[ a_{4u} = \frac{k_u L}{D}, \quad a_{4v} = \frac{k_v L}{D}, \quad a_{4t} = \frac{k_{trans} L}{D}, \]

\[ a_{5u} = \frac{2.303 [H^+]_o}{\beta_{rbc}}, \quad a_{5v} = \frac{2.303 [H^+]_o}{\beta_{pl}}, \]

\[ a_{6u} = \frac{C_{chem,rbc}}{[O_{2}]_o}, \quad a_{6v} = \frac{C_{chem,rbc}}{4 [CO_{2}]_o}, \quad a_{6w} = \frac{K^* f_{water}}{[H^+]_o}, \quad a_{7} = \frac{(S)}{v_{rbc}} T_{tot} K_A, \]

\[ a_{8v1} = \frac{D_{CO_{2,pl}} L}{A r_r^2 (1-b)^2}, \quad a_{8v2} = \frac{D_{CO_{2,pl}} L}{A r_r^2 (1-b)^2}, \]

\[ a_{8w1} = \frac{D_{Hpl} L}{A r_r^2 (1-b)^2}, \quad a_{8w2} = \frac{D_{Hpl} L}{A r_r^2 (1-b)^2}, \]

\[ a_{8r1} = \frac{D_{HCO_{3,pl}} L}{A r_r^2 (1-b)^2}, \quad a_{8r2} = \frac{D_{HCO_{3,pl}} L}{A r_r^2 (1-b)^2}, \]

\[ a_{9u} = \frac{k_u L}{A}, \quad a_{9v} = \frac{k_v L}{A}, \quad a_{9t} = \frac{k_{trans} L}{A}, \]

\[ d_1 = \frac{[H^+]_o [HCO_{3}^-]_o}{K_1 [CO_{2}]_o}, \quad d_2 = \frac{[O_{2}]_o}{[H^+]_o}, \quad d_3 = \frac{[CO_{2}]_o}{[H^+]_o}, \quad d_4 = \frac{[CO_{2}]_o}{[HCO_{3}^-]_o}, \]

\[ d_5 = \frac{[HCO_{3}^-]_o}{[Cl^-]_o}, \quad d_6 = K_A [Cl^-]_o, \quad d_7 = \frac{K_A [HCO_{3}^-]_o^2}{[Cl^-]_o}, \quad d_8 = K_A [HCO_{3}^-]_o, \quad d_9 = \frac{K^*}{K_1}, \]

\[ u_{ext} = \frac{\alpha_{O_{2,rbc}} P_{O_{2,ext}}}{[O_{2}]_o}, \quad v_{ext} = \frac{\alpha_{CO_{2,rbc}} P_{CO_{2,ext}}}{[CO_{2}]_o}. \]
6.1.5 Parameter Values

In order to simulate $O_2/CO_2$ transport and $pH$ regulation by blood, various parameters which are summarized in Table 6.2 must be known or evaluated. Whenever possible, the parameters are based on experimental observations. Where no data is available, estimates are made from existing correlations and theory. When necessary, the values of the parameters found in the literature were corrected for temperature, protein concentration or ionic strength of the medium. A detailed discussion on the evaluation and selection parameters is given in this section.

6.1.5.a Geometrical Parameters

Consider first the geometrical parameters which are listed in Table 6.2. The specification of RBC characteristic dimensions is well established; for the most part, values that are typical of recent work are selected (Nair et al., 1989). The dimensions of the artificial membrane oxygenator come from experimental determinations.

Table 6.2: Values of parameters used in the numerical computations for the discrete model.

<table>
<thead>
<tr>
<th>(a) Geometrical parameters</th>
<th>Red blood cell</th>
<th>Artificial membrane oxygenator</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s$</td>
<td>Red cell surface area</td>
<td>Inner radius of the artificial membrane tube</td>
</tr>
<tr>
<td>$(s)_v^{rbc}$</td>
<td>Surface to volume ratio of the RBC</td>
<td>$r_o$</td>
</tr>
<tr>
<td>$r_{rbc}$</td>
<td>Maximum thickness of the RBC</td>
<td>Outer radius of the artificial membrane tube</td>
</tr>
<tr>
<td>$r_{rbc}$</td>
<td>Radius of the RBC</td>
<td>$d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$L$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163x10^{-8} cm$^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 $\mu m$</td>
</tr>
</tbody>
</table>
Table 6.2: continued

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{heme, rbc}$</td>
<td>Total heme concentration in the RBC</td>
<td>20.8 mM</td>
</tr>
<tr>
<td>$[DPG]$</td>
<td>Intracellular concentration of DPG</td>
<td>6.1 mM</td>
</tr>
<tr>
<td>$f_{water}$</td>
<td>Water fraction of RBC volume</td>
<td>0.72</td>
</tr>
<tr>
<td>$T_{tot}$</td>
<td>Total number of anion transporters per RBC</td>
<td>$1 \times 10^6$</td>
</tr>
<tr>
<td>$\beta_{bc}$</td>
<td>Intracellular buffer capacity</td>
<td>54.5 mM H⁺/pH</td>
</tr>
<tr>
<td>$\alpha_{O_2, rbc}$</td>
<td>Bunsen solubility coefficient for O₂ in RBC</td>
<td>Table 6.3</td>
</tr>
<tr>
<td>$\alpha_{CO_2, rbc}$</td>
<td>Bunsen solubility coefficient for CO₂ in RBC</td>
<td>Table 6.4</td>
</tr>
<tr>
<td>$\beta_{pl}$</td>
<td>Extracellular buffer capacity</td>
<td>5.5 mM H⁺/pH</td>
</tr>
<tr>
<td>$\alpha_{O_2, pl}$</td>
<td>Bunsen solubility coefficient for O₂ in plasma</td>
<td>Table 6.3</td>
</tr>
<tr>
<td>$\alpha_{CO_2, pl}$</td>
<td>Bunsen solubility coefficient for CO₂ in plasma</td>
<td>Table 6.4</td>
</tr>
<tr>
<td>$D_{O_2, pl}$</td>
<td>Diffusion coefficient for O₂ in the plasma</td>
<td>Table 6.5</td>
</tr>
<tr>
<td>$D_{CO_2, pl}$</td>
<td>Diffusion coefficient for CO₂ in the plasma</td>
<td>Table 6.6</td>
</tr>
<tr>
<td>$D_{H^+, pl}$</td>
<td>Diffusion coefficient for H⁺ in the plasma</td>
<td>Tables 6.8, 6.9</td>
</tr>
<tr>
<td>$D_{HCO_3^-, pl}$</td>
<td>Diffusion coefficient for HCO₃⁻ in the plasma</td>
<td>Tables 6.8, 6.9</td>
</tr>
<tr>
<td>$D_{Cl^-, pl}$</td>
<td>Diffusion coefficient for Cl⁻ in the plasma</td>
<td>Tables 6.8, 6.9</td>
</tr>
<tr>
<td>$K_{O_2}$</td>
<td>O₂ permeability of the artificial membrane</td>
<td></td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>CO₂ permeability of the artificial membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Artificial membrane oxygenator</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2: continued

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{50}$</td>
<td>Hill equilibrium parameter</td>
<td>Equation (2.2)</td>
</tr>
<tr>
<td>$n$</td>
<td>Hill equation parameter</td>
<td>2.6</td>
</tr>
<tr>
<td>$S_{min}$</td>
<td>Scaling factors of the modified Easton model</td>
<td>0.12, 0.96</td>
</tr>
<tr>
<td>$S_{max}$</td>
<td>at which maximum slope for modified Easton model occurs</td>
<td>20.7 mmHg</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Rate constant of the modified Easton model</td>
<td>Equation (2.7)</td>
</tr>
</tbody>
</table>

Carbamino-hemoglobin equilibrium relationship

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\alpha}$</td>
<td>Association constant for CO$_2$-binding to the $\alpha$-chain of hemoglobin</td>
<td>$650 \text{ M}^{-1}$ (@ 37 °C)</td>
</tr>
<tr>
<td>$\lambda_{\beta}$</td>
<td>Association constant for CO$_2$-binding to the $\beta$-chain of hemoglobin</td>
<td>$240 \text{ M}^{-1}$ (@ 37 °C)</td>
</tr>
<tr>
<td>$K_{DPG}$</td>
<td>Association constant for DPG and hemoglobin</td>
<td>$1.5 \times 10^3 \text{ M}^{-1}$ - $5 \times 10^2$ (@ 37 °C)</td>
</tr>
</tbody>
</table>

Carbon dioxide hydration-dehydration reactions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K'$</td>
<td>Acid dissociation constant for H$_2$CO$_3$</td>
<td>Table 2.2</td>
</tr>
<tr>
<td>$K_1$</td>
<td>Acid dissociation constant for H$_2$CO$_3$</td>
<td>Table 2.2</td>
</tr>
<tr>
<td>$k_u$</td>
<td>CO$_2$ hydration rate constant</td>
<td>Table 2.2</td>
</tr>
<tr>
<td>$k_v$</td>
<td>H$_2$CO$_3$ dehydration rate constant</td>
<td>Table 2.2</td>
</tr>
<tr>
<td>$\hat{A}_{pol}$</td>
<td>Carbonic anhydrase activity in the plasma</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Anion exchange kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{trans}$</td>
<td>Translocation rate constant</td>
<td>Table 5.8</td>
</tr>
<tr>
<td>$K_A$</td>
<td>Equilibrium association rate constant</td>
<td>Table 5.8</td>
</tr>
</tbody>
</table>
6.1.5.b Physical Parameters

C_{heme,rbc}, [DPG], f_{water}, T_{tot}, \beta_{rbc} and \beta_{pl}

Let's start with the total heme concentration in the RBC which may be calculated from the hemoglobin density within the red cell; in this study, value of 20.8 mM is used. Normal cellular DPG concentration and fraction of water content in RBC are 6.1 mM and 0.72, respectively (Meldon, 1984). The total number of anion
transporters is reported to be $= 1 \times 10^6$ per RBC (Gunn et al., 1973). The intracellular buffering capacity is calculated using data of Rossi-Bernardi and Roughton (1967). They reported that for an 8.8 mM hemoglobin solution, $\beta$ is approximately 23 (mM base)/(pH unit) for either oxygenated or deoxygenated hemoglobin; thus, $\beta_{rbc}$ is calculated to be $= 54.5$ (mM base)/(pH unit). The buffer power of plasma proteins is one-tenth the value of the intracellular buffering capacity (Davenport, 1969); therefore, $\beta_{pl}$ is $= 5.5$ (mM base)/(pH unit).

$\alpha_{O_2,rbc}$, $\alpha_{O_2,pl}$, $\alpha_{CO_2,rbc}$ and $\alpha_{CO_2,pl}$

The solubilities for most of gases of physiological interest are available in the literature. The solubilities of O$_2$ and CO$_2$ in water, plasma and concentrated hemoglobin solution, which is approximately equivalent to the RBC interior, are listed in Tables 6.3 and 6.4.

Table 6.3: Solubility coefficients for oxygen in water (and/or saline), plasma and hemoglobin solution with equivalent heme concentration as that in RBC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>$\alpha_{O_2,H_2O}$</th>
<th>$\alpha_{O_2,pl}$</th>
<th>$\alpha_{O_2,rbc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($\alpha_{O_2,\text{saline}}$)</td>
<td>($10^{-6}$ M/mmHg)</td>
<td>($10^{-6}$ M/mmHg)</td>
</tr>
<tr>
<td>Christoforides and Hedley-Whyte</td>
<td>1.67</td>
<td>1.41</td>
<td>1.51</td>
</tr>
<tr>
<td>Altman and Dittmer</td>
<td>1.66 [1.60]</td>
<td>1.41 [1.34]</td>
<td>1.51</td>
</tr>
<tr>
<td>Sendroy et al.</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Albritton</td>
<td>---</td>
<td>---</td>
<td>1.46</td>
</tr>
</tbody>
</table>

$\dagger$ Value of $\alpha_{O_2,\text{saline}}$ is obtained or estimated for the condition of isotonic saline with a concentration of 0.15 N.
Table 6.4: Solubility coefficients for carbon dioxide in water (and/or saline), plasma and hemoglobin solution with equivalent heme concentration as that in RBC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>$\alpha_{CO2,H2O}$ [(\alpha_{CO2,saline})](^\dagger) (10(^{-5}) M/mmHg)</th>
<th>$\alpha_{CO2,pl}$ (10(^{-5}) M/mmHg)</th>
<th>$\alpha_{CO2,rbc}$ (10(^{-5}) M/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altman and Dittmer</td>
<td>4.31 3.26</td>
<td>4.00 2.98</td>
<td>3.84 2.58</td>
</tr>
<tr>
<td>[4.36] [3.23]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albritton</td>
<td>--- ---</td>
<td>4.10 3.12</td>
<td>3.37 2.55</td>
</tr>
<tr>
<td>Mochizuki and Kagawa</td>
<td>--- ---</td>
<td>--- 3.08</td>
<td>--- 2.62</td>
</tr>
</tbody>
</table>

\(^\dagger\) Value of $\alpha_{CO2,saline}$ is obtained or estimated for the condition of isotonic saline with a concentration of 0.15 N.

**$D_{O2,pl}$ and $D_{CO2,pl}$**

In this study, the flux ($N_i$) of any molecular species ($O_2$ or $CO_2$) is defined via binary diffusivity ($D_i$), that is neglecting any diffusive coupling effect,

$$N_i = -D_i \nabla [C_i]$$  \( (6.48) \)

where $[C_i]$ is the dissolved concentration of either $O_2$ or $CO_2$. Spaeth and Friedlander (1967) and Kreuzer (1970) gave a comprehensive list of experimentally determined values of $O_2$ diffusion coefficients in distilled water, serum protein solutions and hemoglobin solutions at 25 °C and 37 °C. The mean values of these measured quantities are summarized in Table 6.5.
Table 6.5: The diffusivity of O\(_2\) in water, plasma and concentrated hemoglobin solution.

<table>
<thead>
<tr>
<th></th>
<th>(D_{O_2,H_2O}) (cm(^2)/sec)</th>
<th>(D_{O_2,pl}) (cm(^2)/sec)</th>
<th>(D_{O_2,35g%Hb}) (cm(^2)/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(25,^\circ C)</td>
<td>2.15x10(^{-5})</td>
<td>1.73x10(^{-5})</td>
<td>0.74x10(^{-5})</td>
</tr>
<tr>
<td>(37,^\circ C)</td>
<td>2.75x10(^{-5})</td>
<td>2.24x10(^{-5})</td>
<td>0.95x10(^{-5})</td>
</tr>
</tbody>
</table>

The value for \(D_{O_2,pl}\) in Table 6.5 is given for normal human plasma. Plasma contains 8% or 9% protein by weight. Due to a wide variety of substances present in plasma and the normal variations from person to person, it is difficult to establish representative properties for plasma. Various investigators (summarized by Kreuzer (1970); Goldstick and Fatt (1970)) added bovine serum albumin to isotonic solution at the same total protein concentration as that of human plasma. When the resulting diffusion coefficient is plotted versus total protein concentration, an almost linear relationship is found. The value for \(D_{O_2,35g%Hb}\) is given for the concentrated hemoglobin solution which is assumed to be equivalent to that of RBC interior. A plot of \(D_{O_2}\) in protein solutions against protein concentration and a similar plot of \(D_{O_2}\) against hemoglobin concentration at 25 \(^\circ C\) are given in Kreuzer (1970). The effect of electrolytes on the diffusivity of O\(_2\) in water was studied by Goldstick and Fatt (1970). Their conclusion was that saline does have a different diffusivity than distilled water. However, at isotonic concentrations, it was only about 3% lower than that for water. The difference increases drastically, though, as a molarity of 1.0 is approached.

Diffusion coefficients for CO\(_2\) in water and plasma as reported by Altman and Dittmer (1971) Gros and Moll (1974) are summarized in Table 6.6.
Table 6.6: The diffusivity of CO₂ in water and plasma.

<table>
<thead>
<tr>
<th>Authors</th>
<th>$D_{CO2,H2O}$ (cm²/sec)</th>
<th>$D_{CO2,pl}$ (cm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>Altman and Dittmer</td>
<td>1.85x10⁻⁵</td>
<td>2.55x10⁻⁵</td>
</tr>
<tr>
<td>Gros and Moll</td>
<td>1.74x10⁻⁵</td>
<td>---</td>
</tr>
</tbody>
</table>

+ Plasma protein concentration of 33g% methemoglobin.
* Plasma protein concentration of 11g% albumin.

The dependence of the diffusivity of O₂ and CO₂ on the protein concentration has been shown to be simply explainable on the basis of the geometry and water space in the protein solution (Gros and Moll, 1971). The diffusion coefficients for O₂ and CO₂ in plasma protein solutions are reduced as compared to their values in water; values of $D_{O2,pl}/D_{O2,H2O}$ and $D_{CO2,pl}/D_{CO2,H2O}$ for different plasma protein concentration is summarized in Table 6.7. These values are taken from different sources, and the result suggests that the dependence of $D_{O2}$ and $D_{CO2}$ on the protein concentration is similar; i.e., $D_{O2,pl}/D_{O2,H2O} = D_{CO2,pl}/D_{CO2,H2O}$ in the range of plasma protein concentrations.

Table 6.7: Comparison of $D_{O2,pl}/D_{O2,H2O}$ and $D_{CO2,pl}/D_{CO2,H2O}$ for different plasma protein concentrations at $T=25$ °C.

<table>
<thead>
<tr>
<th>plasma protein (wt)</th>
<th>$D_{O2,pl}/D_{O2,H2O}$</th>
<th>$D_{CO2,pl}/D_{CO2,H2O}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5 g%</td>
<td>0.85⁺</td>
<td>---</td>
</tr>
<tr>
<td>11.0 g%</td>
<td>0.79⁺</td>
<td>---</td>
</tr>
<tr>
<td>30.0 g%</td>
<td>0.45⁺ 0.36*</td>
<td>--- 0.78§</td>
</tr>
<tr>
<td>34.0 g%</td>
<td>0.42⁺</td>
<td>0.45*</td>
</tr>
</tbody>
</table>

⁺ denotes value obtained from Kreuzer (1970).
* denotes value obtained from Altman and Dittner (1971).
§ denotes value obtained from Gros and Moll (1974).
concentrations considered. Altman and Dittmer reported a value of 0.46 for $D_{CO_2,pl}^{(a)}$ at 37 °C which is in close agreement as that reported for 25 °C. Since extensive studies of the dependence of diffusivities on protein concentration at 37 °C is not available in the literature, it is assumed that the same reduction as at 25 °C holds.

Since diffusivity measurements were sometimes taken at temperatures other than the physiological temperature, adjustments sometimes are necessary before the values for diffusivities of various species can be used in a transport model. It is a substantial alteration of experimental results and therefore needs to be considered in detail. One way to express the diffusion coefficient as a function of temperature is the Stokes-Einstein correction (Bird et al., 1960):

$$\frac{T}{D_i \mu} = \text{constant}$$

(6.49)

where $D_i$ is the diffusivity of species i at temperature $T$ and $\mu$ is the viscosity of the solvent. Unfortunately the viscosity is also a function of temperature. Although the viscosity of water at different temperatures is available, that for other fluids is not. However because plasma is an aqueous based solution, its temperature corrections would be expected to be approximately the same as that for water. Spaeth and Friedlander (1967) tested the applicability of this relationship for temperature corrections of experimental data in the range of 25 °C to 37 °C. Their results indicated reasonable agreement with the theory. Their result showed that $D_{O_2,H_2O}$ increases by a factor of ≈1.3 and $D_{O_2,pl}$ increases by a factor of ≈1.34 as $T$ increases from 25 to 37 °C. A second means of correcting the diffusivity coefficient was discussed by Roughton (1959). Over a small temperature range, the diffusivity can be
approximated by a linear correction, usually given as a percent change of diffusivity for each degree change in temperature \((T)\):

\[
\frac{D_{i,T}}{D_{i,T_0}} = (1 + \Gamma (T - T_0))
\]  

(6.50)

where \(D_{i,T_0}\) is the diffusivity of specie \(i\) at reference temperature \(T_0\) and \(\Gamma\) is given as 2.5\% \((\degree C)^{-1}\) for gases in aqueous protein solutions. For temperatures in the range of 20 to 40 \degree C, it gives close agreement with the Stokes-Einstein correlation. For the change between 25 and 37 \degree C, Equation (6.50) gives a correction factor of 1.34.

\(\tilde{D}_{H_{\text{pt}}}, \tilde{D}_{HCO_3\text{pt}},\) and \(\tilde{D}_{Cl\text{pt}}\)

The situation is remarkably more complex when, as invariably turns out to be the case, the diffusivities of ionic species need to be estimated. The reason for the additional complexities in estimation of the diffusivities for ionic species is that the diffusion of different ions is coupled in the sense that the diffusive flux of any one ion depends on the concentration gradients of all ions in solution. In principle, one would have to set up the diffusion reaction equations taking this into account; the equations would then be coupled not only through the reaction terms but also the diffusion term (i.e., one would have both chemical and ionic coupling). For these reasons, only limited data exist in the literature for the diffusivities of the ionic species. Hashitani and Kigoshi (1965) measured the diffusivity of \(HCO_3^-\) in aqueous solutions as \(1.09 \times 10^{-5}\) cm\(^2\)/sec at 25 \degree C. Benn et al. (1975) used the Nernst-Hartley equation (Horvath, 1985), which is applicable to diffusion of a single anion-cation pair at infinite dilution, to calculate diffusivity of \(HCO_3^-\) in weak acid solutions as \(1.18 \times 10^{-5}\) cm\(^2\)/sec at 25 \degree C.

In the diffusion of any particular ion \(i\) in a mixture of ions the operative factors are: its concentration gradient (here expressed as \(\nabla [C_i]\)), its electrical valency \((Z_i)\), its
fractional resistance to movement (expressed as the reciprocal of its equivalent conductivity \( \lambda_i \)) and the electrical gradient \( (\nabla \psi) \) produced by and a function of all ions present. These fundamental principles are quantitatively expressed as follows (Bird et al., 1960)

\[
N_i = -D_i \left[ \nabla [C_i] + Z_i [C_i] \nabla \psi \right]
\]  
(6.51)

where values of \( D_i \) are available in the literature for common ions and can be evaluated from measurements of electrical conductance. Astarita et al. (1983) considered the problem and developed the following approximation scheme. The electrical capacity of liquids is so low that even a minor imbalance in the local charge concentration would result in very large peaks of electrical potential. Hence, to all practical purpose, the solution is electrically neutral everywhere.

\[
\sum_i Z_i [C_i] = 0
\]  
(6.52)

The flux of ion \( i \) carries a current proportional to \( Z_i N_i \). Although in an electrochemical cell the total net current is non-zero; in an aqueous solutions, as in blood, there is no net electrical current and, therefore,

\[
\sum_i Z_i N_i = 0
\]  
(6.53)

Substitution of Equation (6.51) into (6.53) and algebraic rearrangement yields the electrical potential gradient
\[ \nabla \psi = - \frac{\sum Z_i D_i \nabla [C_i]}{\sum Z_i^2 D_i [C_i]} \]  

(6.54)

Notice that if all \( D_i \)s are equal, the gradient of electrical potential is zero, and Equation (6.51) reduces to the ordinary diffusion equation for non-ionic species. However, if the ionic diffusivities are sufficiently different from each other to generate a non-negligible electrical potential gradient, then the \( Z_i [C_i] \nabla \psi \) term may provide strong ionic coupling of diffusion. The approximate procedure that Astarita et al. (1983) tried to develop was to estimate an "apparent" diffusivity of ionic species \( \tilde{D}_i \) defined by

\[ N_i = - \tilde{D}_i \nabla [C_i] \]  

(6.55)

Consider the case of plasma. The ionic species to be considered are \( Na^+, Cl^- \) and \( HCO_3^- \); \( K^+, H^+, OH^-, H_2PO_4^- \) and \( HPO_4^{2-} \) are not considered because their concentrations are known to be negligible as compared to that of \( Na^+, Cl^- \) and \( HCO_3^- \). The electroneutrality condition requires

\[ [Na^+]_{pl} = [Cl^-]_{pl} + [HCO_3^-]_{pl} \]  

(6.56)

so that the electrical potential gradient is calculated from Equation (6.54) as

\[ \nabla \psi = - \frac{(D_{HCO_3^{2-}} - D_{Cl,pl}) \nabla [HCO_3^-]_{pl} - (D_{Na,pl} - D_{Cl,pl}) \nabla [Na^+]_{pl}}{(D_{HCO_3^{2-}} - D_{Cl,pl}) [HCO_3^-]_{pl} + (D_{Na,pl} + D_{Cl,pl}) [Na^+]_{pl}} \]  

(6.57)

However, an additional condition is imposed by the physical fact that the flux of \( Na^+ \) needs necessarily to be zero. Therefore, from the appropriate form of Equation (6.51),
\[ \nabla \psi = - \frac{\nabla [Na^+]_{pl}}{[Na^+]_{pl}} \]

Substitution of Equations (6.57) and (6.58) into appropriate form of Equation (6.51) and rearrangement yields the apparent diffusivities

\[
\tilde{D}_{Cl,pl} = D_{Cl,pl} \frac{2 (D_{Na,pl} + D_{HCO_3,pl} - D_{Cl,pl}) [Na^+]_{pl}}{(D_{HCO_3,pl} - D_{Cl,pl}) [HCO_3^-]_{pl} + (2 D_{Na,pl} + D_{HCO_3,pl} - D_{Cl,pl}) [Na^+]_{pl}}
\]

\[
\tilde{D}_{HCO_3,pl} = D_{HCO_3,pl} \frac{2 D_{Na,pl} [Na^+]_{pl}}{(D_{HCO_3,pl} - D_{Cl,pl}) [HCO_3^-]_{pl} + 2 D_{Na,pl} [Na^+]_{pl}}
\]

with the equivalent diffusivity \( \tilde{D}_{Na,pl} \) being zero, since flux of Na\(^+\) is zero although \( \nabla [Na^+]_{pl} \) is not.

For an ideal electrolyte solution, \( D_i \)'s can be calculated directly from their equivalent conductivities using an equation derived by Nernst (Horvath, 1985):

\[
D_i^0 = \lambda_i^0 \left( \frac{RT}{|Z_i|F^2} \right)
\]

where

- \( D_i^0 \) is the diffusion coefficient of ion i in infinitely dilute solution, cm\(^2\)/sec.
- \( \lambda_i^0 \) is the equivalent conductance of ion i in infinitely dilute solution, cm\(^2\) Ω\(^{-1}\) mole\(^{-1}\).
- \( R \) is the universal gas constant (=8.316 Joules mole\(^{-1}\) K\(^{-1}\)).
- \( T \) is the absolute temperature, K.
- \( Z_i \) is the electrical valence of ion i.
- \( F \) is the Faraday number (=96,500 coulombs mole\(^{-1}\)).
Because $D_i^0$ is the value of the diffusion coefficient of ion $i$ when completely released from other ions and not under the influence of a potential gradient. As a result, corrections have to be made for the effects of protein concentration and ionic strength. As discussed above, the diffusion coefficients of $O_2$ and $CO_2$ in a 8.5 g/100 ml plasma protein solution are roughly 85% of their respective values in water (Table 6.7). As a rough estimate, it seems reasonable to assume that the protein affects the diffusion of other small molecules in a similar way. Since no experimental data is available, it is assumed that the same reduction holds for the diffusivities of the ionic species. However, the corrections dealing with the effects of non-ideal electrolyte solutions on the diffusivities of the ionic species are more complicated. A brief discussion on the relationship between the non-ideality of electrolyte solutions and the charge and concentrations is given below.

The concentration dependence of diffusion coefficient in dilute aqueous electrolyte solutions is given by the following expression:

$$D_i = D_i^0 \left(1 + \frac{d \ln \gamma_i}{d \ln [C_i]} \right)$$

(6.62)

where $\gamma_i$ is the activity coefficient of species $i$. The variation of $D_i$ with concentration is due to the thermodynamic term $(d \ln \gamma_i / d \ln [C_i])$. Generally, the methods for calculating activity coefficients in multicomponent systems are built upon the calculation of activity coefficients of single electrolyte solutions (e.g., Debye-Hückel’s law; a complete review of the various approaches for dealing with single electrolyte solutions is given in Horvath, 1985) together with the application of mixing rules. Plasma is a combination of both strong (e.g., $NaCl \leftrightarrow Na^+ + Cl^-$) and weak electrolytes (e.g., $NaHCO_3 \leftrightarrow Na^+ + HCO_3^-\)$. Therefore, plasma is by definition to contain both strong electrolytes (compounds which nearly dissociates to its maximally
charged constituent ions when placed in aqueous solution) and complexing electrolytes (compounds which form a non-trivial quantities of non-maximally charged complexes). There is a vast literature dealing with the theory of multicomponent electrolyte solutions which is compiled in *Handbook of Aqueous Electrolyte Thermodynamics* by Zemaitis et al. (1986). For instance, Bromley's method (Zemaitis et al., 1986) would be applicable for use with this system; the ionic activity coefficient of ion *i* is:

\[
\ln \gamma_i = -\frac{C_{DH} Z_i^2 \sqrt{I}}{1 + \sqrt{I}} + \sum_j B_{ij} Z_i^2 [C_j]
\]  

(6.63)

where

- \(C_{DH}\) is the Debye-Hückel constant.
- \(I\) is the ionic strength, and \(I = 0.5 \sum_i [C_i] Z_i^2\).
- \(Z_{i,j} = \frac{Z_i + Z_j}{2}\).
- \(B_{ij} = \frac{(0.06 + 0.6 B_\sigma |Z_i Z_j|) + B_\sigma}{1 + \frac{1.5}{|Z_i Z_j|} I^2}\).

and \(B_\sigma\) is the Bromley interaction term. Bromley's expression is applicable here due to the summation term; the species may be any of the complexes in the solution. Using this approach to estimate the non-ideality of the electrolyte solution would be reasonable from a fundamental standpoint. However, because of the lack of complete and accurate values for the interaction terms, the practicality of using this correction is diminished.

For a first order approximation, Equation (6.61) au. plasma protein correction factor, listed in Table 6.7, are used to calculate \(D_{Cl, pl}\), \(D_{HCO_3, pl}\) and \(D_{Na, pl}\); in other
words, the non-ideality of the solution is ignored and the thermodynamic term \( d \ln \gamma_i / d \ln C_i \) put equal to zero. Values of the limiting equivalent conductivities in water \( (\lambda_i^0) \), ionic diffusivities at infinite dilute solution \( (D_i^0) \) calculated from Equation (6.61), and apparent diffusivities \( (\tilde{D}_{i,pl}) \) calculated via Equations (6.59) and (6.60) are listed in Tables 6.8 and 6.9 at \( T=25 \, ^\circ\text{C} \) and \( T=37 \, ^\circ\text{C} \), respectively.

In an attempt to correct for the effects of ionic strength, Landolt and Bronstein's experimental data (summarized by Gros and Moll, 1974) on the relation between ionic strength and conductivity of NaHCO\(_3\) were used to estimate the reduction of HCO\(_3^-\) conductivity/diffusivity by the ionic strength. Accordingly, the diffusion coefficient is reduced to 77\% of \( D_{HCO3}^0 \) for \( I_m=0.13 \) (albumin solution with \( pH=7.2 \)), to 73\% of \( D_{HCO3}^0 \) for \( I_m=0.78 \) (albumin solution with \( pH=7.8 \)) where \( I_m \) is the ionic strength calculated using the molality of ions. Hence the apparent diffusivities for different ions in the plasma are calculated as follows: \( D_{i,pl} = 0.75 \cdot 0.85 \cdot D_i^0 \).

Because experimental data is not available, it is then assumed here that the effect of ionic strength and equivalent conductivity of univalent salts such as NaCl and NaHCO\(_3\) are similar. As a result, correction by above equation is also applied to Na\(^+\) and Cl\(^-\). The apparent diffusivities are calculated to be the followings: \( \tilde{D}_{Cl,pl} = 1.03 \times 10^{-5} \) and \( \tilde{D}_{HCO3,pl} = 0.952 \times 10^{-5} \) cm\(^2\)/sec at 25 \, ^\circ\text{C}; and \( \tilde{D}_{Cl,pl} = 1.29 \times 10^{-5} \) and \( \tilde{D}_{HCO3,pl} = 1.25 \times 10^{-5} \) cm\(^2\)/sec at 37 \, ^\circ\text{C}.

6.1.5.c Equilibrium and Kinetic Parameters

Empirical relationships which allow the estimate of ODC parameters of normal human whole blood at both standard and nonstandard conditions were obtained by Samaja et al. (1981) and Buerk and Bridges (1986) who used direct nonlinear regression applied to available literature data; Equation (2.2) for estimating Hill parameter, \( P_{50} \) (Samaja et al., 1981) or Equation (2.7) for estimating Easton parameter, \( \kappa \) (Buerk and Bridges, 1986). Parameter values for the carbamino-
Table 6.8: Estimation of $\tilde{D}_{H,pl}$, $\tilde{D}_{HCO_3,pl}$ and $\tilde{D}_{Cl,pl}$ at 25 °C.

<table>
<thead>
<tr>
<th>Ionic species i</th>
<th>$\lambda_i^o$ † (cm²/Ω-mole)</th>
<th>$D_i^o$ * (cm²/sec)</th>
<th>$D_{i,pl}^o$ ‡ (cm²/sec)</th>
<th>$\tilde{D}_{i, pl}$ § (cm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>350.10</td>
<td>9.28x10⁻⁵</td>
<td>7.89x10⁻⁵</td>
<td>7.89x10⁻⁵</td>
</tr>
<tr>
<td>Na⁺</td>
<td>50.11</td>
<td>1.33x10⁻⁵</td>
<td>1.13x10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>76.34</td>
<td>2.03x10⁻⁵</td>
<td>1.72x10⁻⁵</td>
<td>1.32x10⁻⁵</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>54.50</td>
<td>1.45x10⁻⁵</td>
<td>1.23x10⁻⁵</td>
<td>1.28x10⁻⁵</td>
</tr>
</tbody>
</table>

† The values of $\lambda_i^o$ are obtained from Table 2.10.1 in *Handbook of Aqueous Electrolyte Solutions* (Horvath, 1985).

* The diffusivities of various ions are calculated from Equation (6.61).

‡ The calculated diffusivities are further corrected for the plasma protein concentration (8.5 g%); i.e., $D_{i,pl}^o = 0.85 \times D_i^o$.

§ The apparent or effective ionic diffusivities are calculated via Equations (6.59) and (6.60) where $[Na^+]_{pl} = 134$ mM and $[HCO_3^-]_{pl} = 27$ mM.

Table 6.9: Estimation of $\tilde{D}_{H,pl}$, $\tilde{D}_{HCO_3,pl}$ and $\tilde{D}_{Cl,pl}$ at 37 °C.

<table>
<thead>
<tr>
<th>Ionic species i</th>
<th>$\lambda_i^o$ † (cm²/Ω-mole)</th>
<th>$D_i^o$ * (cm²/sec)</th>
<th>$D_{i,pl}^o$ ‡ (cm²/sec)</th>
<th>$\tilde{D}_{i, pl}$ § (cm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>404.76</td>
<td>1.12x10⁻⁴</td>
<td>9.52x10⁻⁵</td>
<td>9.52x10⁻⁵</td>
</tr>
<tr>
<td>Na⁺</td>
<td>64.26</td>
<td>1.77x10⁻⁵</td>
<td>1.50x10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>95.88</td>
<td>2.65x10⁻⁵</td>
<td>2.25x10⁻⁵</td>
<td>1.73x10⁻⁵</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>68.07</td>
<td>1.88x10⁻⁵</td>
<td>1.60x10⁻⁵</td>
<td>1.66x10⁻⁵</td>
</tr>
</tbody>
</table>

† The values of $\lambda_i^o$ are obtained from Tables 2.10.2 and 2.11.2 in *Handbook of Aqueous Electrolyte Solutions* (Horvath, 1985).

* The diffusivities of various ions are calculated from Equation (6.61).

‡ The calculated diffusivities are further corrected for the plasma protein concentration (8.5 g%); i.e., $D_{i,pl}^o = 0.85 \times D_i^o$.

§ The apparent or effective ionic diffusivities are calculated via Equations (6.59) and (6.60) where $[Na^+]_{pl} = 134$ mM and $[HCO_3^-]_{pl} = 27$ mM.
hemoglobin equilibrium listed in Table 6.2 are reported for human deoxyhemoglobin in the absence of DPG at pH 7.4 and 37 °C. In order to incorporate the oxygen saturation dependence (the Haldane effect) into account, the following information was implemented into Equation (2.10). By drawing data from various publications, Nunn (1987) reported that in the absence of DPG, the carbamino CO₂ bound to oxyhemoglobin is about 30% of that bound to deoxyhemoglobin for various $P_{CO₂}$. In addition, there is a linear relationship between the difference in carbamino carriage and $S$. Therefore, it is proposed here to extrapolate $\phi(S,[DPG])$ from $\phi(S=0,[DPG])$ via the following relationship:

$$\phi(S,[DPG]) = \phi(S=0,[DPG]) (1 - 0.7S) \quad (6.64)$$

where $\phi(S=0,[DPG])$ is calculated using Equation (2.10) with the equilibrium parameters which were determined for stripped deoxyhemoglobin. Values for various rate and equilibrium constants for the CO₂ hydration/dehydration gathered from the literature are synopsized in Table 2.2. Kinetic parameters for anion transporter operating via ping-pong mechanism are summarized in Table 5.8.

6.1.5.d **Hydrodynamic Parameters**

Due to the Fahraeus effect the tube hematocrit, $H_T$, is lower than the discharge hematocrit, $H_D$. The tube hematocrit used in this work is based on the measurements of Gaehgens et al. (1978), who determined tube hematocrit as a function of discharge hematocrit, tube radius and flow rate. The cell-free layer thickness, $\delta$, is calculated from Equation (2.14). The blunting factor, $B$, represents the deviation of velocity profile from the Poiseuille flow parabolic profile; $B$ is known to depend on vessel diameter, hematocrit and shear rate. Because Nair et al.’s (1989) studies showed that their oxygen transport results are not highly sensitive to the value of $B$, a constant
value of $B$ is used in this work. For instance, $B = 0.9$ for vessels with diameters of $\approx 30 \, \mu m$ and $B = 1$ for vessels with diameters of several hundred $\mu m$. The slip constant, $slp$, is the difference in the velocity of the suspending fluid and the particle at a particular point due to the finite size of the particle, and it is a function of particle position and hematocrit. For the sake of simplicity, a constant slip of 0.1 is assumed in this work.

The parameters $h_m$, $m'$, $A$ and $D$ can be obtained by simultaneous solution of equations which describe: the continuity of plasma velocity profile, the overall RBC mass balance, the overall plasma mass balance and the definition of tube hematocrit. The compatibility conditions of the model are integral expressions (Equations (6.65a) - (6.65d)) which when applied to the hematocrit and velocity profiles can be reduced to the closed form expressions given in Equations (6.66a) - (6.66d) (Nair et al., 1989).

Continuity of plasma velocity profile:

$$V_{pl}'(r) = V_{pl}(r)$$  \hspace{1cm} (6.65a)

Overall RBC mass balance which defines $H_D$, the discharge hematocrit:

$$Q \, H_D = \int_0^r 2 \, \pi \, r \, V_{rbc}(r) \, h(r) \, dr$$  \hspace{1cm} (6.65b)

Overall plasma mass balance:

$$Q \, (1 - H_D) = \int_0^r 2 \, \pi \, r \, V_{pl}(r) \, (1 - h(r)) \, dr + \int_{rr}^r 2 \, \pi \, r \, V_{pl}'(r) \, dr$$  \hspace{1cm} (6.65c)

Definition of tube hematocrit, $H_T$, the space average hematocrit at any cross section in the tube:
\[ H_T = \frac{1}{\pi r_c^2} \int_{0}^{r_c} 2\pi r h(r) \, dr \] (6.65d)

Simultaneous solution of above equations yields the followings:

\[ m' = \frac{2(2\varphi - 1)}{1 - \varphi} \] (6.66a)

\[ h_m = \frac{H_T(m' + 2)}{m' b^2} \] (6.66b)

\[ D = \frac{2QH_D\left[1 + \frac{1-H_D}{H_D}(1-slp)\right]}{\pi r_c^2(1-slp)[1 + b^2(1 - B)]} \] (6.66c)

\[ A = \frac{D(1 - Bb^2)}{1 - b^2} \] (6.66d)

where

\[ b = \frac{r_x}{r_c}. \]

\[ \varphi = \frac{2}{B b^2} \left[1 - \frac{1 + b^2(1-B)}{2H_T\left[1 + \frac{1-H_D}{H_D}(1-slp)\right]}\right] \text{ (for } 0.5 < \varphi < 1). \]

### 6.1.5.e System Parameters

All the quantities which are listed under the category of system parameters should be accessible to experimental measurements. Sometimes, only some of the quantities are determined by the investigators. For illustrations, let’s consider Voorhees’ (1976) system where the following quantities are available from experimental determinations: \( T, P_{O_2,\text{ext}}, P_{CO_2,\text{ext}}, P_{O_2,\text{in}}, P_{CO_2,\text{in}}, pH_{\text{pl, in}} \) and total inlet \( CO_2 \) content. As a result, one has to reconstruct the inlet composition for other species.
The inlet composition is assumed to be in equilibrium and uniform. In addition, if \((pH_{rbc,in} - pH_{pl,in})\) is assumed to be a function \([DPG]\) and \(P_{CO2,in}\) and independent of temperature between 25 °C and 37 °C, then the result obtained by Meldon (1984) can be used to estimate \(pH_{rbc,in}\) from the experimentally determined \(pH_{pl,in}\) using Equation (6.67).

\[
pH_{rbc,in} = pH_{pl,in} - \Delta pH
\]

(6.67)

where \(\Delta pH\) is the difference between the \(pH\) in the RBCs from that in the plasma which was calculated by Meldon (1984). Meldon (1984) calculated \(pH_{pl}\) and \(pH_{rbc}\) and other dependent variables from \(P_{CO2}\) and \([DPG]\) in oxygenated whole blood, \textit{in vitro} at 37 °C by applying published data on RBC and plasma buffer capacity, RBC/plasma Donnan distribution, plus CO₂ solubility and reaction equilibrium constants and enforcing fundamental thermodynamic and conservation constraints. Because we are interested in the difference between but not the actual values of the \(pH\) in RBC and plasma, it is then reasonable to neglect the fact that the inlet \(O_2\) saturation of blood samples in Voorhees’ experiments were \(\geq 70\%\) instead of 100%, and the operating temperature was 25 °C instead of 37 °C.

The inlet intracellular and extracellular concentrations of other species can then be calculated as follows.

(a) The dissolved CO₂ is in equilibrium with HCO₃⁻ and H⁺ in both intra- and extracellular phases. Therefore,

\[
\frac{[H^+]_{rbc,in} [HCO_3^-]_{rbc,in}}{[CO_2]_{rbc,in}} = K' f_{water}
\]

(6.68a)
\[
\frac{[H^+]_{pl,in} [HCO_3^-]_{pl,in}}{[CO_2]_{pl,in}} = K'
\] (6.68b)

Normally RBCs contain some 72% water and plasma 94% by volume. Meldon's analysis (1984) which used fundamental physiochemical constraints and equilibrium data established the fact that CO₂-induced osmotic effects, or shift of water between cells and plasma, are small (< 3-5%). Therefore, \( f_{\text{water}} \) is taken to be a constant. In addition, from Table 2.2 it is observed that and reaction and equilibrium constants for CO₂ hydration/dehydration reactions in both buffered saline and plasma are about the same; consequently, no volume correction term is incorporated into Equation (6.68b). Furthermore, because \([CO_2]_{\text{rbc}} = \frac{\alpha_{CO_2,\text{rbc}}}{P_{CO_2}}\) and \([CO_2]_{\text{pl}} = \frac{\alpha_{CO_2,\text{pl}}}{P_{CO_2}}\) the intra- and extracellular HCO₃⁻ concentrations can then be calculated via Equations (6.68a) and (6.68b), respectively.

(b) Intra- and extracellular concentrations of Cl⁻ were not determined in Voorhees' work. In making the calculations, extracellular Cl⁻ concentration under standard condition \((T=37 \, ^{\circ}C, P_{O_2}=100 \, \text{mmHg} \) and \( P_{CO_2}=40 \, \text{mmHg} \)) is used, and intracellular Cl⁻ concentration is then calculated from the Donnan ratio:

\[
\frac{[HCO_3^-]_{\text{rbc,in}}}{[HCO_3^-]_{\text{pl,in}}} = \frac{[Cl^-]_{\text{rbc,in}}}{[Cl^-]_{\text{pl,in}}} = R_GD
\] (6.69)

Although the actual Cl⁻ concentrations are not used, it is assumed that Gibbs Donnan ratio used here will set up the appropriate driving force for the transmembrane transport. The usage of the actual Cl⁻ concentrations here might not be essential because Cl⁻ is not involved in any other chemical reaction but the one-for-one exchange of Cl⁻ for HCO₃⁻; and the Cl⁻ flux is equal and opposite to that of HCO₃⁻ across the RBC membrane. Therefore, it is presumed that within the physiological
concentration range, if the driving force is represented appropriately, then the actual values of Cl\(^-\) should not alter the results on other components appreciably.

(c) Finally, total inlet CO\(_2\) content, \([CO_2]_{\text{total, in}}\), can then be used as a consistency check for the above calculations.

\[
[CO_2]_{\text{total, in}} = [HCO_3^-]_{\text{pl, in}}(1 - H_D) + [HCO_3^-]_{\text{rbc, in}} H_D + \left[ \alpha_{CO_2,rbc} H_D + \alpha_{CO_2,pl} (1 - H_D) \right] PCO_{2, in} + [HbCO_2]_{\text{in}} H_D + [H_2CO_3]_{\text{pl, in}} (1 - H_D) + [H_2CO_3]_{\text{rbc, in}} H_D\] (6.70)

Carbonic acid presence is negligible because both intra- and extracellular \(pH\) in the system are much higher than the \(pK\) of the acid; therefore, it can be neglected from the calculation of \([CO_2]_{\text{total, in}}\).

In the case where the inlet \(pH\) of the blood sample was not available, then the following approach is used to estimate \(pH_{\text{pl}}\) and \(pH_{\text{rbc}}\).

(a) Given the quantity \(PCO_{2, in}\), \([HbCO_2]_{\text{in}}\) can be calculated from Equation (6.71),

\[
[HbCO_2]_{\text{in}} = \left( \frac{C_{\text{heme, rbc}}}{4} \right) \left( \frac{2 \lambda_\alpha \alpha_{CO_2} PCO_{2, in}}{1 + \lambda_\alpha \alpha_{CO_2} PCO_{2, in}} + \frac{2 \lambda_\beta \alpha_{CO_2} PCO_{2, in}}{1 + \lambda_\beta \alpha_{CO_2} PCO_{2, in} + KD_{\text{PG}} [DPG]} \right)
\] (6.71)

(b) From Equations (6.68a) and (6.68b), \([HCO_3^-]_{\text{rbc, in}}\) and \([HCO_3^-]_{\text{pl, in}}\) can be expressed terms of inlet CO\(_2\) and hydrogen ion compositions:

\[
[HCO_3^-]_{\text{rbc, in}} = \frac{K' f_{\text{water}} [CO_2]_{\text{rbc, in}}}{[H^+]_{\text{rbc, in}}} \] (6.72a)

\[
[HCO_3^-]_{\text{pl, in}} = \frac{K' [CO_2]_{\text{pl, in}}}{[H^+]_{\text{pl, in}}} \] (6.72b)
In addition, from Equation (6.67), \([H^+]_{rbc} = 10^{\Delta p H} [H^+]_{pl}\) is obtained.

(c) Substituting Equations (6.71) and (6.72) into (6.70) and utilizing the relationship that \([H^+]_{rbc} = 10^{\Delta p H} [H^+]_{pl}\), an equation with \([H^+]_{pl,in}\) as the unknown is obtained. Rearrange the equation and solve for \([H^+]_{pl,in}\):

\[
[H^+]_{pl,in} = \frac{K' P_{CO2, in} \left[ H_{DF\, water} \alpha_{CO2, rbc} + (1-H_D)\alpha_{CO2, pl} 10^{\Delta p H} \right]}{10^{\Delta p H} \left[ \left[ CO2 \right]_{total, in} - \left[ H_D\alpha_{CO2, rbc} + (1-H_D)\alpha_{CO2, pl} \right] P_{CO2, in} - H_D[HbCO2]_{in} \right]}
\]

(6.73)

### 6.1.6 Numerical Method and Processing of Simulation Results for Comparison of Model Predictions with Experimental Measurements

Solution of the transport equations is complicated by nonlinear reaction equilibrium and kinetic terms and coupling among the equations. Thus all the partial differential equations are solved numerically by the finite element collocation method of Madsen and Sincovec (1975) which uses basic spline polynomials for the spatial discretization. This method was described and evaluated for problems of \(O_2\) transport in capillaries by Baxley and Hellums (1983) and it was shown to be much more efficient than finite difference methods. The numerical solution to the transport equations, subject to the imposed boundary and inlet conditions, provides the concentration profiles for various species as functions of \(r\) and \(z\). The selection of the appropriate physical and chemical parameters is summarized in Table 6.2 for the discrete model which is consisted of Equations (6.1.1) - (6.1.7); and Table 6.10 for the continuum model which is consisted of Equations (3.23) and (4.14).
Table 6.10: Values of parameters used in the numerical computations for the continuum model.

<table>
<thead>
<tr>
<th>Physical properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{O_2}$, effective O$_2$ solubility coefficient</td>
<td>$\alpha_{O_2} = H_D \alpha_{O_2, rbc} + (1 - H_D) \alpha_{O_2, pl}$</td>
</tr>
<tr>
<td>$\alpha_{CO_2}$, effective CO$_2$ solubility coefficient</td>
<td>$\alpha_{CO_2} = H_D \alpha_{CO_2, rbc} + (1 - H_D) \alpha_{CO_2, pl}$</td>
</tr>
<tr>
<td>$D_{O_2}$, effective O$_2$ diffusion coefficient</td>
<td>$D_{O_2} = H_D D_{O_2, rbc} + (1 - H_D) D_{O_2, pl}$</td>
</tr>
<tr>
<td>$D_{CO_2}$, effective CO$_2$ diffusion coefficient</td>
<td>$D_{CO_2} = H_D D_{CO_2, rbc} + (1 - H_D) D_{CO_2, pl}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Equilibrium parameters for approximating CO$_2$ dissociation curve</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{eq}^{\dagger}$, pseudo equilibrium constant</td>
<td>$K_{eq} = 54.2 ; @ ; 25 ; ^{\circ}C^{*}; ; 78.6 ; @ ; 37 ; ^{\circ}C^{\ddagger}$</td>
</tr>
<tr>
<td>$\beta^{\dagger}$, apparent buffering capacity of the blood</td>
<td>$\beta = 31.7 ; @ ; 25 ; ^{\circ}C^{*}; ; 32.5 ; @ ; 37 ; ^{\circ}C^{\ddagger}$</td>
</tr>
</tbody>
</table>

$^{\dagger}$ The values of $K_{eq}$ and $\beta$ used to simulate Voorhees' experiments are given in the legend for each run; $K_{eq}=$[dimensionless] and $\beta=$[mM H$^+$/pH].

$^{*}$ Basis: $T=25 \; ^{\circ}C$, $H_D=45\%$, $P_{CO_2}=56$ mmHg, $[CO_2]_{total}=24.4$ mM, $pH_{pl}=7.501$ (Voorhees, 1976).

$^{\ddagger}$ Basis: $T=37 \; ^{\circ}C$, $H_D=45\%$, $P_{CO_2}=46$ mmHg, $[CO_2]_{total}=23.2$ mM, $pH_{pl}=7.37$ (Voorhees, 1976).

Gas transport predictions are obtained for the experimental runs conducted by Voorhees (1976) as the numerical solutions for either Equations (6.1.1) - (6.1.7) listed in Table 6.1 for the discrete model and Equations (3.23) and (4.14) for the continuum model. For the discrete model, mixed mean concentrations of species inside the RBC ($[C_i]_{mm,rbc}$), in the plasma ($[C_i]_{mm,pl}$) and in the whole blood ($[C_i]_{mm,wb}$) are calculated by Equations (6.74a), (6.74b) and (6.74c), respectively.

\[
[C_i]_{mm,rbc}(z) = \frac{1}{\pi r_z^2 H_T \langle V \rangle_{rbc}} \int_0^{r_c} 2 \pi r h(r) V_{rbc}(r) [C_i]_{rbc}(r, z) \, dr
\]  
(6.74a)

\[
[C_i]_{mm,pl}(z) = \frac{1}{\pi r_z^2 (1 - H_T) \langle V \rangle_{pl}} \int_0^{r_r} 2 \pi r (1 - h(r)) V_{pl}(r) [C_i]_{pl}(r, z) \, dr
\]
\[ + \frac{1}{\pi r_c^2 (1 - H(r)} \langle V \rangle_{pl} \int_{r_c}^r 2 \pi r V_{pl}'(r) [C_i]_{pl}(r,z) \, dr \]  

(6.74b)

\[ [C_i]_{mm,wb}(z) = \frac{1}{\pi r_c^2 \langle V \rangle} \int_0^{r_c} 2 \pi r h(r) V_{rb}(r) [C_i]_{rb}(r,z) \, dr \]

\[ + \frac{1}{\pi r_c^2 \langle V \rangle} \int_0^{r_r} 2 \pi r (1 - h(r)) V_{pl}(r) [C_i]_{pl}(r,z) \, dr \]

\[ + \frac{1}{\pi r_c^2 \langle V \rangle} \int_{r_c}^r 2 \pi r V_{pl}'(r) [C_i]_{pl}(r,z) \, dr \]  

(6.74c)

For the continuum model, the mixed mean concentration of species \( i \) in the tube (\([C_i]_{mm}\)) is evaluated as

\[ [C_i]_{mm}(z) = \frac{1}{\pi r_c^2} \int_0^{r_c} 4 \pi r \left[ 1 - \left( \frac{L}{r_c} \right)^2 \right] [C_i](r,z) \, dr \]

(6.75)

The comparison with Voorhees’ data are presented as changes in both total O\(_2\) content, \([O_2]_{total}\), and total CO\(_2\) content, \([CO_2]_{total}\), versus transit time. Here the transit time is defined as the ratio of active exchange length to the average fluid velocity in the tube; i.e., transit time = \( r_c^2 L / Q \). The total content change of O\(_2\) or CO\(_2\) is calculated from

\[ \Delta [C_i]_{total} = [C_i]_{total, out} - [C_i]_{total, in} \]

where

\[ \Delta [C_i]_{total} \] is the total content change of species \( i \).

\([C_i]_{total, out}\) and \([C_i]_{total, in}\) are the total concentrations of species \( i \) at \( z=L \) and \( z=0 \), respectively.
For the discrete model, \([O_2]_{total}\) and \([CO_2]_{total}\) are computed as the follows:

\[
[O_2]_{total} = H_D \cdot C_{heme, rbc} \cdot S_{mm, rbc} + [O_2]_{mm, wb}
\] (6.77a)

\[
[CO_2]_{total} = [HCO_3^-]_{mm, wb} + [CO_2]_{mm, wb} + H_D \cdot [HbCO_2]_{mm, rbc}
\] (6.77b)

For the continuum model, these quantities are calculated as

\[
[O_2]_{total} = C_{heme} \cdot S_{mm} + [O_2]_{mm}
\] (6.78a)

\[
[CO_2]_{total} = [CO_2]_{reacted, mm} + [CO_2]_{mm}
\] (6.78b)

6.2 Results and Discussion

6.2.1 Comparison with Voorhees’ Membrane Oxygenator Data

Experimental results by Voorhees (1976) were used for comparison with the calculations from the models. In this work, freshly drawn human blood was equilibrated to the desired inlet conditions at \(= 25 \, ^\circ\text{C}\) and then perfused through an artificial membrane oxygenator. Simultaneous \(O_2\) and \(CO_2\) transfer were measured in a single straight permeable tube constructed of dimethyl silicone. Two basic transfer units were used by Voorhees (1976); and the geometrical parameters for those units were I.D. = 1.47 mm, O.D. = 1.96 mm, \(L = 179 \, \text{cm}\) and I.D. = 1.57 mm, O.D. = 3.17 mm, \(L = 179 \, \text{cm}\). The \(O_2\) and \(CO_2\) permeabilities in dimethyl-silicone are \(2.5 \times 10^{-9}\) and \(1.5 \times 10^{-8} \, \text{M cm}^{2}/\text{sec/mmHg}\), respectively (Dorson et al., 1971). Minimum and maximum experimental values were: inlet \(O_2\) saturation from 69 to 81\%, inlet \(CO_2\) tension from 27.9 to 59.7 mmHg, inlet plasma \(pH\) from 7.0 to 7.7, and hematocrit between 42 and 57\%. Oxygenation experiments, in which RBC suspensions were
taking up O₂ and eliminating CO₂ to the environment, were carried out by suffusing the environmental gas space with almost pure oxygen ($P_{O₂, ext} = 698-718$ mmHg and $P_{CO₂, ext} = 0$ mmHg). Deoxygenation experiments, in which RBC suspensions were delivering O₂ to the environment and taking up CO₂, were conducted by suffusing the external gas chamber with a N₂-CO₂ gas mixture ($P_{O₂, ext} = 0$ mmHg and $P_{CO₂, ext} = 115$ mmHg).

Data reported by Voorhees were taken with care in measuring not only most of the relevant blood gas parameters (e.g., $T$, $H_D$, $S$, $P_{O₂}$, $P_{CO₂}$, and $[CO₂]_{total}$) for the inlet and outlet blood samples, but also the biochemical status of each sample (such as $K_{eq}$ and $\beta$ which are parameters for characterizing the CO₂ dissociation curve, O₂ consumption, and/or $\Delta pH$ and $\Delta P_{CO₂}$). However, it should be mentioned that hemoglobin O₂ affinity and cooperativity which are required for describing the O₂ dissociation curve were not reported. As a result, in order to compare the theoretical predictions with Voorhees’ experimental data, these equilibrium parameters are calculated based on Equation (2.2) and the temperature coefficient ($\partial \log P_{O₂}/\partial T$) of whole blood which was tabulated by Zwart et al. (1984).

Figures 6.1a - 6.9a show both the total O₂ content change and CO₂ content change in comparison to the predictions generated by both the discrete and continuum models. It can be seen from the left-hand plots of these figures that both models are equally successful in predicting the O₂ transport over the conditions studied, except for the deoxygenation case (Figure 6.9a) where the continuum model under predicts the amount of transport by a substantial fraction. The discrete model consistently predicts more transport than the continuum model. As mentioned previously in Section 4.3.2, for large vessels, the discrete model reduces to the continuum model for describing O₂ transport. Therefore, the difference between the O₂ transfer rates predicted by these models can be partially explained by the fact that $D_{O₂, pl}$ used ($D_{O₂, pl} = 1.60 \times 10^{-5}$
Figure 6.1a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 22 °C with \( H_D = 0.42 \), \( P_{50,in} = 10.1 \) mmHg, \( K_{eq} = 257.9 \), \( \beta = 31.8 \) mM H⁺/pH, \( pH_{pl,in} = 7.66 \), \( P_{O_2,in} = 17.5 \) mmHg (corresponding to 7.08 mM in \([O_2]_{total}\)), \( P_{CO_2,in} = 27.9 \) mmHg (corresponding to 31.1 mM in \([CO_2]_{total}\)), \( P_{O_2,ext} = 718 \) mmHg and \( P_{CO_2,ext} = 0 \) mmHg. The left panel gives the total \( O_2 \) content change as a function of transit time; and the right panel, the total \( CO_2 \) content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (——) the discrete model and (----) the continuum model.
Figure 6.2a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 22 °C with \( H_D = 0.46 \), \( P_{50, in} = 13.6 \) mmHg, \( K_{eq} = 105.0 \), \( \beta = 32.6 \) mM H⁺/pH, \( pH_{pl, in} = 7.28 \), \( P_{O_2, in} = 18.5 \) mmHg (corresponding to 7.08 mM in \([O_2]_{total}\)), \( P_{CO_2, in} = 63.3 \) mmHg (corresponding to 32.5 mM in \([CO_2]_{total}\)), \( P_{O_2, ext} = 718 \) mmHg and \( P_{CO_2, ext} = 0 \) mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (-------) the discrete model and (- - - -) the continuum model.
Figure 6.3a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 22 °C with $H_D=0.43$, $P_{S0,in}=17.6$ mmHg, $K_{eq}=22.9$, $\beta=27.8$ mM H⁺/pH, $pH_{pl,in}=6.98$, $P_{O2,in}=23.0$ mmHg (corresponding to 6.63 mM in $[O_2]_{total}$), $P_{CO2,in}=57.7$ mmHg (corresponding to 16.3 mM in $[CO_2]_{total}$), $P_{O2,ext}=720$ mmHg and $P_{CO2,ext}=0$ mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (———) the discrete model and (-----) the continuum model.
**Figure 6.4a:** Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 27 °C with $H_D=0.42$, $P_{50,in}=19.4$ mmHg, $K_{eq}=54.2$, $\beta=32.1$ mM H⁺/pH, $pH_{pl,in}=7.19$, $P_{O_2,in}=25.0$ mmHg (corresponding to 6.04 mM in $[O_2]_{total}$), $P_{CO_2,in}=59.6$ mmHg (corresponding to 24.4 mM in $[CO_2]_{total}$), $P_{O_2,ex}=698$ mmHg and $P_{CO_2,ex}=0$ mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (-----) the discrete model and (-----) the continuum model.
Figure 6.5a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 24 °C with $H_D=0.57$, $P_{50,in}=15.3$ mmHg, $K_{eq}=58.0$, $\beta=40.2$ mM H⁺/pH, $pH_{pl,in}=7.28$, $P_{O2,in}=25.0$ mmHg (corresponding to 8.84 mM in $[O_2]_{total}$), $P_{CO2,in}=51.8$ mmHg (corresponding to 27.5 mM in $[CO_2]_{total}$), $P_{O2,ext}=704$ mmHg and $P_{CO2,ext}=0$ mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (-----) the discrete model and (- - - - -) the continuum model.
Figure 6.6a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 25 °C with \( H_D = 0.43, P_{50, in} = 16.8 \text{ mmHg}, K_{eq} = 46.1, \beta = 32.4 \text{ mM H}^+/\text{pH}, P_{H^+, in} = 7.23, P_{O_2, in} = 22.7 \text{ mmHg} \) (corresponding to 6.56 mM in \([O_2]_{\text{total}}\)), \( P_{CO_2, in} = 54.4 \text{ mmHg} \) (corresponding to 23.1 mM in \([CO_2]_{\text{total}}\)), \( P_{O_2, ext} = 708 \text{ mmHg} \) and \( P_{CO_2, ext} = 0 \text{ mmHg} \). The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (---) the discrete model and (- - - -) the continuum model.
Figure 6.7a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.57-mm-diameter membrane tube at 26 °C with \( H_D = 0.43 \), \( P_{50, \text{in}} = 16.7 \) mmHg, \( K_{eq} = 45.7 \), \( \beta = 31.8 \) mM \( \text{H}^+ / \text{pH} \), \( pH_{pl, \text{in}} = 7.32 \), \( P_{O_2, \text{in}} = 22.4 \) mmHg (corresponding to 6.39 mM in \([O_2]_{\text{total}}\)), \( P_{CO_2, \text{in}} = 40.9 \) mmHg (corresponding to 19.2 mM in \([CO_2]_{\text{total}}\)), \( P_{O_2, \text{ext}} = 707 \) mmHg and \( P_{CO_2, \text{ext}} = 0 \) mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (——) the discrete model and (- - - - -) the continuum model.
Figure 6.8a: Comparison of models for oxygenation accompanied by CO₂ elimination of RBC suspensions flowing in a 1.57-mm-diameter membrane tube at 25 °C with \( H_D = 0.43 \), \( P_{50,in} = 20.6 \) mmHg, \( K_{eq} = 24.2 \), \( \beta = 31.3 \) mM H⁺/pH, \( pH_{pl,in} = 6.99 \), \( P_{O2,in} = 32.0 \) mmHg (corresponding to 6.57 mM in \([O_2]_{total}\)), \( P_{CO2,in} = 52.0 \) mmHg (corresponding to 17.1 mM in \([CO_2]_{total}\)), \( P_{O2,ext} = 709 \) mmHg and \( P_{CO2,ext} = 0 \) mmHg. The left panel gives the total O₂ content change as a function of transit time; and the right panel, the total CO₂ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (-----) the discrete model and (- - - -) the continuum model.
Figure 6.9a: Comparison of models for deoxygenation accompanied by CO$_2$ uptake of RBC suspensions flowing in a 1.47-mm-diameter membrane tube at 25 °C with $H_D$=0.43, $P_{O_2, in}$=17.6 mmHg, $K_{eq}$=40.3, $\beta$=31.5 mM H$^+/p$H, $pH_{pl,in}$=7.18, $P_{O_2, in}$=22.5 mmHg (corresponding to 6.38 mM in [O$_2$_total]), $P_{CO_2, in}$=53.9 mmHg (corresponding to 21.3 mM in [CO$_2$_total]), $P_{O_2, ext}$=0 mmHg and $P_{CO_2, ext}$=115 mmHg. The left panel gives the total O$_2$ content change as a function of transit time; and the right panel, the total CO$_2$ content change. Data points: means of experiments from Voorhees (1976). Curves: theoretical simulation curves for the same conditions; (——) the discrete model and (-----) the continuum model.
Figure 6.10: Comparison of the \( \Delta[CO_2]_{total} \) results calculated by the continuum (CM) and discrete (DM via different values of \( \hat{A}_{pl} \)) models for RBC suspensions flowing in a 1.47-mm-diameter membrane tube with discharge hematocrit of 42% at 25 °C. The left panel gives the comparison for an oxygenation (and CO₂ elimination) case: \( P_{50, in}=16.8 \) mmHg, \( K_{eq}=46.1 \), \( \beta=32.4 \) mM H⁺/pH, \( pH_{pl,in}=7.23 \), \( P_{O_2, in}=22.7 \) mmHg, \( P_{CO_2, in}=54.4 \) mmHg, \( P_{O_2, ext}=708 \) mmHg and \( P_{CO_2, ext}=0 \) mmHg. Curves: theoretical simulation of the situations; (———) both CM and DM via \( \hat{A}_{pl}=6500 \), (-----) DM via \( \hat{A}_{pl}=100 \), (— — —) DM via \( \hat{A}_{pl}=1 \). The right panel gives the comparison for a deoxygenation (and CO₂ uptake) case: \( P_{50, in}=17.6 \) mmHg, \( K_{eq}=40.3 \), \( \beta=31.5 \) mM H⁺/pH, \( pH_{pl,in}=7.18 \), \( P_{O_2, in}=22.5 \) mmHg, \( P_{CO_2, in}=53.9 \) mmHg, \( P_{O_2, ext}=0 \) mmHg and \( P_{CO_2, ext}=115 \) mmHg. Curves: theoretical simulation of the situations; (———) CM, (-----) DM via \( \hat{A}_{pl}=6500 \), (— — —) DM via \( \hat{A}_{pl}=1 \).
cm²/sec @ T=25 °C and H_D=42%; discrete model) is higher than the value of \( D_{O2} \)
\( (D_{O2,pt}=1.25\times10^{-5} \text{ cm}^2/\text{sec} @ T=25 \text{ °C and } H_D=42\% ; \text{ continuum model}) \). Another cause contributing to this difference is due to the fact that the Bohr effect is taken into consideration in the discrete model but not in the continuum model. For the oxygenation runs (Figures 6.1a - 6.8a), differences of no more than 0.51 mM in \( \Delta [O_2]_{total} \) were observed between simulation results generated by the two models; this difference amounts to \( \approx 15\% \) of the change in \( [O_2]_{total} \). However, for the deoxygenation case (Figure 6.9a), the continuum model predicts far less transport than the discrete model; and the maximum difference between the models is 0.41 mM which amounts to \( \approx 53\% \) of the change in \( [O_2]_{total} \). The explanation of this observation is that the Bohr effect plays a more important role during deoxygenation run (more details in Section 6.2.2a). The maximum deviations between the experimental data and the theoretical curves generated by and the discrete and continuum models are 0.35 mM and 0.71 mM in \( \Delta [O_2]_{total} \), respectively. The algebraic average deviations for any one curve generated by the discrete and continuum models do not exceed 0.15 mM and 0.25 mM in \( \Delta [O_2]_{total} \), respectively.

More importantly, from the right-hand plots of Figures 6.1a - 6.9a, it is clear that the discrete model represents an improvement in accuracy for predicting CO₂ transport over the continuum model. In all the cases, the continuum model predicts more transport than experimentally observed. The maximum deviations between the experimental data and the theoretical curves generated by the discrete and continuum models are 0.8 mM and 6.7 mM in \( \Delta [CO_2]_{total} \), respectively. The algebraic average deviation from the experimental data for any one curve generated by the discrete and continuum models do not exceed 0.37 mM and 2.4 mM in \( \Delta [CO_2]_{total} \), respectively.

A major factor in the success of the discrete model for predicting CO₂ transfer is due to the incorporation of the slow CO₂ hydration/ dehydration reactions in the
plasma. This conclusion is substantiated and demonstrated by Figure 6.10 where upon setting $\hat{A}_{pl}=6500$ the amount of CO$_2$ transfer predicted by the discrete model approaches that predicted by the continuum model for both oxygenation and deoxygenation cases. $\hat{A}_{pl}=6500$ represents the situation where carbonic anhydrase activity equivalent to that of the RBC interior is available to the plasma. For these runs, differences ranging from 1.0 to 7.0 mM in $\Delta$[CO$_2$]$_{total}$ were observed between simulation results generated by the two models at transit time of $\approx$ 5.8 min.

Figures 6.1b - 6.9b present the mixed mean values of $P_{O2}$, $S$, $P_{CO2}$ and intracellular [HbCO$_2$] for three different flow rates under the experimental conditions of runs 1 to 9; and Figures 6.1c - 6.9c show that for intra- and extracellular $pH$, $[HCO_3^-]_{rbc}$ and $[HCO_3^-]_{pl}$. As was expected the larger the flow rate, the smaller the change in these quantities. These figures may be useful for calculations of membrane oxygenator performance so as to ensure proper blood oxygenation and CO$_2$ stripping. Sizing of the oxygenator is important with respect to minimization of priming volume as well as with respect to assurance of proper O$_2$ and CO$_2$ transfer. Over-design of the oxygenator brings blood oxygenation to the 95-100% O$_2$ saturation range. However, along with the above effect, over design also leads to the possibilities of blood $P_{O2}$ becoming intolerably great and excessive CO$_2$ removal. Intolerably high $P_{O2}$ raises the concern with oxygen toxicity; and improper removal of CO$_2$ is undesirable because of the associated disturbance of acid/base balance. For illustrations, Figures 6.5b and 6.5c show that at flow rate of 0.52 ml/min 95% O$_2$ saturation, which is normal arterial level, is reached at about $z=110$ cm. The corresponding $P_{O2}$ of 160 mmHg is high, and this is due to the high $P_{O2,ext}$ used in the study. On the other hand, the normal arterial level of CO$_2$ tension is about 40 mmHg and plasma $pH$ is about 7.4. These values are reached at $z=80$ cm. For an existing equipment, these results would mean
Figure 6.1b: Mixed mean values of $O_2$ tension, $O_2$ saturation, $CO_2$ tension and intracellular HbCO$2$ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T$=22 °C, $H_D=0.42$, $P_{50,in}=10.1$ mmHg, $K_{eq}=257.9$, $\beta=31.8$ mM H$^+$/pH, $pH_{pl,in}=7.66$, $P_{O2,in}=17.5$ mmHg, $P_{CO2,in}=27.9$ mmHg, $P_{O2,ext}=718$ mmHg and $P_{CO2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (———) $Q=2.08$ ml/min, (-----) $Q=1.04$ ml/min, (— — — — —) $Q=0.52$ ml/min.
Figure 6.1c: Mixed mean values of intra- and extracellular $pH$ and intra- and extracellular $HCO_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T=22$ °C, $H_D=0.42$, $P_{50, in}=10.1$ mmHg, $k_{eq}=257.9$, $\beta=31.8$ mM $H^+/pH$, $pH_{pl, in}=7.66$, $P_{O_2, in}=17.5$ mmHg, $P_{CO_2, in}=27.9$ mmHg, $P_{O_2, ext}=718$ mmHg and $P_{CO_2, ext}=0$ mmHg. Simulation curves: generated via the discrete model; (-----) $Q=2.08$ ml/min, (- - - -) $Q=1.04$ ml/min, (---) $Q=0.52$ ml/min.
Figure 6.2b: Mixed mean values of $O_2$ tension, $O_2$ saturation, $CO_2$ tension and intracellular $HbCO_2$ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D. = 1.47 mm, O.D. = 1.95 mm, $T = 22 \, ^\circC$, $H_D = 0.46$, $P_{50, in} = 13.6 \, mmHg$, $K_{eq} = 105.0$, $\beta = 32.6 \, mM \, H^+/pH$, $pH_{pi, in} = 7.28$, $P_{O2, in} = 18.5 \, mmHg$, $P_{CO2, in} = 63.3 \, mmHg$, $P_{O2, ext} = 718 \, mmHg$ and $P_{CO2, ext} = 0 \, mmHg$. Simulation curves: generated via the discrete model; (-----) $Q = 2.08 \, ml/min$, (----) $Q = 1.04 \, ml/min$, (---) $Q = 0.52 \, ml/min$. 
Figure 6.2c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO$_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T$=22 °C, $H_D$=0.46, $P_{50,in}$=13.6 mmHg, $K_{eq}$=105.0, $\beta$=32.6 mM H$^+$/pH, $pH_{pl,in}$=7.28, $P_{O_2,in}$=18.5 mmHg, $P_{CO_2,in}$=63.3 mmHg, $P_{O_2,ext}$=718 mmHg and $P_{CO_2,ext}$=0 mmHg. Simulation curves: generated via the discrete model; (-----) $Q$=2.08 ml/min, (----) $Q$=1.04 ml/min, (---) $Q$=0.52 ml/min.
Figure 6.3b: Mixed mean values of $O_2$ tension, $O_2$ saturation, $CO_2$ tension and intracellular $HbCO_2$ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D. = 1.47 mm, O.D. = 1.95 mm, $T$ = 22 °C, $H_D$ = 0.43, $P_{50, in}$ = 17.6 mmHg, $K_{eq}$ = 2.29, $\beta$ = 27.8 mM H+/$pH$, $pH_{p,l,in}$ = 6.98, $P_{O2, in}$ = 23.0 mmHg, $P_{CO2, in}$ = 57.7 mmHg, $P_{O2, ext}$ = 720 mmHg and $P_{CO2, ext}$ = 0 mmHg. Simulation curves: generated via the discrete model; (——) $Q$ = 2.08 ml/min, (-----) $Q$ = 1.04 ml/min, (— — —) $Q$ = 0.52 ml/min.
Figure 6.3c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO$_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D. = 1.47 mm, O.D. = 1.95 mm, $T=22$ °C, $H_D=0.43$, $P_{50,in}=17.6$ mmHg, $K_{eq}=22.9$, $\beta=27.8$ mM H$^+/\text{pH}$, $pH_{pl,in}=6.98$, $P_{O2,in}=23.0$ mmHg, $P_{CO2,in}=57.7$ mmHg, $P_{O2,ext}=720$ mmHg and $P_{CO2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (----) $Q=2.08$ ml/min, (-- -- --) $Q=1.04$ ml/min, (---) $Q=0.52$ ml/min.
Figure 6.4b: Mixed mean values of $O_2$ tension, $O_2$ saturation, $CO_2$ tension and intracellular Hb$CO_2$ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T=27$ °C, $H_D=0.42$, $P_{50,in}=19.4$ mmHg, $K_{eq}=54.2$, $\beta=32.1$ mM H$^+$/pH, $pH_{pl,in}=7.19$, $P_{O2,in}=25.0$ mmHg, $P_{CO2,in}=59.6$ mmHg, $P_{O2,ext}=698$ mmHg and $P_{CO2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (-------) $Q=2.08$ ml/min, (- - - -) $Q=1.04$ ml/min, (-----) $Q=0.52$ ml/min.
Figure 6.4c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO$_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D. = 1.47 mm, O.D. = 1.95 mm, $T = 27$ °C, $H_D = 0.42$, $P_{50, in} = 19.4$ mmHg, $K_{eq} = 54.2$, $\beta = 32.1$ mM H$^+$/pH, $pH_{pl,in} = 7.19$, $P_{O2,in} = 25.0$ mmHg, $P_{CO2,in} = 59.6$ mmHg, $P_{O2,ext} = 698$ mmHg and $P_{CO2,ext} = 0$ mmHg. Simulation curves: generated via the discrete model: (———) $Q = 2.08$ ml/min, (- - - -) $Q = 1.04$ ml/min, (— - —) $Q = 0.52$ ml/min.
Figure 6.5b: Mixed mean values of O₂ tension, O₂ saturation, CO₂ tension and intracellular HbCO₂ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, T=24 °C, \( H_D=0.57 \), \( P_{50,in}=15.3 \) mmHg, \( K_{eq}=58.0 \), \( \beta=40.2 \) mM H⁺/pH, \( pH_{i,in}=7.28 \), \( P_{O2,in}=25.0 \) mmHg, \( P_{CO2,in}=51.8 \) mmHg, \( P_{O2,ext}=704 \) mmHg and \( P_{CO2,ext}=0 \) mmHg. Simulation curves: generated via the discrete model; (---) \( Q=2.08 \) ml/min, (- - - -) \( Q=1.04 \) ml/min, (---) \( Q=0.52 \) ml/min.
Figure 6.5c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO$_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T=24$ °C, $H_D=0.57$, $P_{50,in}=15.3$ mmHg, $K_{eq}=58.0$, $\beta=40.2$ mM H$^+$/pH, $pH_{pl,in}=7.28$, $P_{O2,in}=25.0$ mmHg, $P_{CO2,in}=51.8$ mmHg, $P_{O2,ext}=704$ mmHg and $P_{CO2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (-----) $Q=2.08$ ml/min, (- - - -) $Q=1.04$ ml/min, (-----) $Q=0.52$ ml/min.
Figure 6.6b: Mixed mean values of O₂ tension, O₂ saturation, CO₂ tension and intracellular HbCO₂ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, T=25 °C, \( H_D=0.43 \), \( P_{50,in}=16.8 \text{ mmHg} \), \( K_{eq}=46.1 \), \( \beta=32.4 \text{ mM H}^+/\text{pH} \), \( pH_{pi,in}=7.23 \), \( P_{O2,in}=22.7 \text{ mmHg} \), \( P_{CO2,in}=54.4 \text{ mmHg} \), \( P_{O2,ext}=708 \text{ mmHg} \) and \( P_{CO2,ext}=0 \text{ mmHg} \). Simulation curves: generated via the discrete model; (-----) \( Q=2.08 \text{ ml/min} \), (-----) \( Q=1.04 \text{ ml/min} \), (-----) \( Q=0.52 \text{ ml/min} \).
Figure 6.6c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO$_3^-$ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, $T=25 \, ^\circ C$, $H_D=0.43$, $P_{50,in}=16.8 \, \text{mmHg}$, $K_{eq}=46.1$, $\beta=32.4 \, \text{mM H}^+/\text{pH}$, $pH_{pi,in}=7.23$, $P_{O2,in}=22.7 \, \text{mmHg}$, $P_{CO2,in}=54.4 \, \text{mmHg}$, $P_{O2,ext}=708 \, \text{mmHg}$ and $P_{CO2,ext}=0 \, \text{mmHg}$. Simulation curves: generated via the discrete model; ($———$) $Q=2.08 \, \text{ml/min}$, ($-\cdots-\cdots$) $Q=1.04 \, \text{ml/min}$, ($-\cdot-\cdot$) $Q=0.52 \, \text{ml/min}$. 
Figure 6.7b: Mixed mean values of O\textsubscript{2} tension, O\textsubscript{2} saturation, CO\textsubscript{2} tension and intracellular HbCO\textsubscript{2} concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.57 mm, O.D.=3.18 mm, T=26 °C, $H_D=0.43$, $P_{S0,in}=16.7$ mmHg, $K_{eq}=45.7$, $\beta=31.8$ mM H$^+$/pH, $pH_{PI,in}=7.32$, $P_{O2,in}=22.4$ mmHg, $P_{CO2,in}=40.9$ mmHg, $P_{O2,ext}=707$ mmHg and $P_{CO2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (———) $Q=2.08$ ml/min, (- - - - -) $Q=1.04$ ml/min, (— —) $Q=0.52$ ml/min.
Figure 6.7c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO₃⁻ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.57 mm, O.D.=3.18 mm, T=26 °C, $H_D=0.43$, $P_{50,in}=16.7$ mmHg, $K_{eq}=45.7$, $\beta=31.8$ mM H⁺/pH, $pH_{pl,in}=7.32$, $P_{O_2,in}=22.4$ mmHg, $P_{CO_2,in}=40.9$ mmHg, $P_{O_2,ext}=707$ mmHg and $P_{CO_2,ext}=0$ mmHg. Simulation curves: generated via the discrete model; (——) $Q=2.08$ ml/min, (- - - - ) $Q=1.04$ ml/min, (--- - - - ) $Q=0.52$ ml/min.
Figure 6.8b: Mixed mean values of O$_2$ tension, O$_2$ saturation, CO$_2$ tension and intracellular HbCO$_2$ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.57 mm, O.D.=3.18 mm, T=25 °C, $H_D$=0.43, $P_{50,in}$=20.6 mmHg, $K_{eq}$=24.2, $\beta$=31.3 mM H$^+$/pH, $pH_{pt,in}$=6.99, $P_{O2,in}$=32.0 mmHg, $P_{CO2,in}$=52.0 mmHg, $P_{O2,ext}$=709 mmHg and $P_{CO2,ext}$=0 mmHg. Simulation curves: generated via the discrete model; (———) $Q$=2.08 ml/min, (- - - -) $Q$=1.04 ml/min, (—- . —) $Q$=0.52 ml/min.
**Figure 6.8c:** Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO₃⁻ concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.57 mm, O.D.=3.18 mm, T=25 °C, \(H_D=0.43\), \(P_{50,in}=20.6\) mmHg, \(K_{eq}=24.2\), \(\beta=31.3\) mM H⁺/pH, \(pH_{pl,in}=6.99\), \(P_{O2,in}=32.0\) mmHg, \(P_{CO2,in}=52.0\) mmHg, \(P_{O2,ext}=709\) mmHg and \(P_{CO2,ext}=0\) mmHg. Simulation curves: generated via the discrete model; (——) \(Q=2.08\) ml/min, (· · · · · ·) \(Q=1.04\) ml/min, (— · — ·) \(Q=0.52\) ml/min.
Figure 6.9b: Mixed mean values of O₂ tension, O₂ saturation, CO₂ tension and intracellular HbCO₂ concentration at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, T=25 °C, \( H_D=0.43 \), \( P_{50,in}=17.6 \) mmHg, \( K_{eq}=40.3 \), \( \beta=31.5 \) mM H⁺/pH, \( pH_{pi,in}=7.18 \), \( P_{O2,in}=22.5 \) mmHg, \( P_{CO2,in}=53.9 \) mmHg, \( P_{O2,ext}=0 \) mmHg and \( P_{CO2,ext}=115 \) mmHg. Simulation curves: generated via the discrete model; (———) \( Q=2.08 \) ml/min, (- - - - -) \( Q=1.04 \) ml/min, (- - - - -) \( Q=0.52 \) ml/min.
Figure 6.9c: Mixed mean values of intra- and extracellular pH and intra- and extracellular HCO\textsubscript{3}\textsuperscript{-} concentrations at different axial positions along the membrane oxygenator. Experimental conditions: I.D.=1.47 mm, O.D.=1.95 mm, T=25 °C, \( H_D=0.43 \), \( P_{50,in}=17.6 \) mmHg, \( K_{eq}=40.3 \), \( \beta=31.5 \) mM, \( H^+/{\text{pH}} \), \( pH_{pl,in}=7.18 \), \( P_{O2,in}=22.5 \) mmHg, \( P_{CO2,in}=53.9 \) mmHg, \( P_{O2,ext}=0 \) mmHg and \( P_{CO2,ext}=115 \) mmHg. Simulation curves: generated via the discrete model: (-----) \( Q=2.08 \) ml/min, (- - - -) \( Q=1.04 \) ml/min, (--- ---) \( Q=0.52 \) ml/min.
that in order to achieve normal arterial blood composition certain operating parameters need to be altered. For instance, one can either increase $P_{CO_2, in}$ or $P_{CO_2, ext}$ to prevent disproportionate stripping of CO$_2$. For design purpose, the results indicate that in these operating conditions the limiting design phenomenon is the O$_2$ transport and not the CO$_2$ removal.

6.2.2 Analysis of the Effects of Several Important Determinants on O$_2$/CO$_2$ Exchange in Microvessels

Coupled O$_2$ and CO$_2$ transport are determined by the complex interaction of multiple factors. In this section, several of the major determinants which are of particular significance in determining the gas transport are considered. These factors include the influences of the Bohr and Haldane effects, the rate of CO$_2$ hydration/dehydration reactions within the plasma, the kinetics of RBC anion exchange, and the effects of discharge hematocrit. In order to quantify the specific effects of alterations in the various factors mentioned, the discrete model developed in Section 6.1 is used. The computations were performed for the artificial membrane tube systems of Voorhees (1976) and Boland et al. (1987) and for both oxygenation and deoxygenation cases. Table 6.11 summarizes the values chosen for the system parameters. For each case considered, except for the parameters specifically noted, all other physical and chemical parameters are chosen to represent the normal and typical values (see Table 6.2). Although the absolute values of various quantities of the numerical solutions depend on the choice of these values, in most cases the observations on the various trends are found not to be critical.
Table 6.11: Description of the two types of artificial membrane tubes considered and values of the system parameters used in computations.

<table>
<thead>
<tr>
<th>Two types of artificial membrane tubes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1. Voorhees’ membrane oxygenator system: a 1.47-mm-diameter and 179-cm-active length tube which is embedded in a concentric film with an outer diameter of 1.95 mm.</td>
</tr>
<tr>
<td>Type 2. Boland’s membrane oxygenator system: a 27-μm-diameter and 5-mm-active length tube which is embedded in a 170-μm-thickness slab.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Values of the system parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Situation</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Discharge hematocrit</td>
</tr>
<tr>
<td>Inlet composition of RBC suspension</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Composition of the external gas space</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

6.2.2.a Effects of the Bohr and Haldane Phenomena

Interactions of $O_2$ and $CO_2$ exchange are included in the discrete model. To study the importance of these interactions, the exchange of $O_2$ and $CO_2$ are calculated in the presence and absence of simultaneous transfer of the other gas. The influence of the Bohr effect on oxygenation is shown for the Type 1 and 2 membrane tubes in
Figures 6.11 and 6.12, respectively; on deoxygenation, Figures 6.13 and 6.14. The left-hand plots of Figures 6.11 - 6.14 present the results of total O$_2$ content change versus transit time; and the right-hand plots give the change in mixed mean $P_{O_2}$ and O$_2$ saturation. These figures illustrate the fact that when no CO$_2$ transfer is allowed, the calculated total O$_2$ content changes are less than in the case of simultaneous O$_2$/CO$_2$ exchange. The calculated total O$_2$ content changes for these cases are summarized in Table 6.12. These calculations indicated that the Bohr effect accounts for about 20% of the total O$_2$ exchange for the deoxygenation case; and less than 10% for the oxygenation case. The Bohr effect is more significant for the deoxygenation case as compared to the oxygenation case; and it becomes increasingly more important for smaller vessels as compared to the larger vessels.

Table 6.12: Influence of the Bohr effect on O$_2$ transfer for both oxygenation and deoxygenation cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>$\Delta [O_2]<em>{total} = [O_2]</em>{total}(z=L) - [O_2]_{total}(z=0)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O$_2$ transport alone</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)‡</td>
<td>8.09 mM</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 µm)*</td>
<td>5.90 mM</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>-1.28 mM</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 µm)*</td>
<td>-2.10 mM</td>
</tr>
</tbody>
</table>

‡ Type 1 membrane tube: $Q=12.5$ ml/hr.
* Type 2 membrane tube: $Q=9$ µl/hr.
† Values in parentheses denote the % increase in the amount of total O$_2$ transfer which is calculated as:

\[
\% \uparrow = \frac{\Delta [O_2]_{total(O_2/CO_2~transfer)} - \Delta [O_2]_{total(O_2~transfer~alone)}}{\Delta [O_2]_{total(O_2/CO_2~transfer)}}.
\]
Figure 6.11: Influence of the Bohr effect on rate of $O_2$ uptake by human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total $O_2$ content change as a function of transit time ($[O_2]_{total,in}=1.09$ mM). Curves: theoretical simulation of the situations; (——) $O_2$ transfer in the presence of $CO_2$ exchange, (---) $O_2$ transfer in the absence of $CO_2$ exchange. The right panel gives both changes in mixed mean $P_{O_2}$ and $O_2$ saturation as functions of transit time ($P_{O_2,in}=14.0$ mmHg, $S_{in}=0.12$). Curves: theoretical simulation of the situations; (——, left-hand scale) and (— —, right-hand scale) $O_2$ transfer in the presence of $CO_2$ exchange; (---, left-hand scale) and (— ——, right-hand scale) $O_2$ transfer in the absence of $CO_2$ exchange.
Figure 6.12: Influence of the Bohr effect on rate of O₂ uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total O₂ content change as a function of transit time (\([O_2]_{\text{total, in}}=1.09\) mM). Curves: theoretical simulation of the situations; (-----) O₂ transfer in the presence of CO₂ exchange, (-------) O₂ transfer in the absence of CO₂ exchange. The right panel gives both changes in mixed mean \(P_{O_2}\) and O₂ saturation as functions of transit time (\(P_{O2, \text{in}}=14.0\) mmHg, \(S_{\text{in}}=0.12\)). Curves: theoretical simulation of the situations; (----, left-hand scale) and (-----, right-hand scale) O₂ transfer in the presence of CO₂ exchange; (-----, left-hand scale) and (--------, right-hand scale) O₂ transfer in the absence of CO₂ exchange.
Figure 6.13: Influence of the Bohr effect on rate of $O_2$ release from human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total $O_2$ content change as a function of transit time ($[O_2]_{total, in}$=8.43 mM). Curves: theoretical simulation of the situations; (——) $O_2$ transfer in the presence of $CO_2$ exchange, (- - - -) $O_2$ transfer in the absence of $CO_2$ exchange. The right panel gives both changes in mixed mean $P_{O_2}$ and $O_2$ saturation as functions of transit time ($P_{O_2, in}$=68.1 mmHg, $S_{in}$=0.94). Curves: theoretical simulation of the situations; (——, left-hand scale) and (---, right-hand scale) $O_2$ transfer in the presence of $CO_2$ exchange; (- - - - , left-hand scale) and (·······, right-hand scale) $O_2$ transfer in the absence of $CO_2$ exchange.
Figure 6.14: Influence of the Bohr effect on rate of O₂ release from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total O₂ content change as a function of transit time ([O₂]_{total,in}=8.43 mM). Curves: theoretical simulation of the situations; (———) O₂ transfer in the presence of CO₂ exchange, (---------) O₂ transfer in the absence of CO₂ exchange. The right panel gives both changes in mixed mean P_{O₂} and O₂ saturation as functions of transit time (P_{O₂,in}=68.1 mmHg, S_{in}=0.94). Curves: theoretical simulation of the situations; (—— — , left-hand scale) and (— ---, right-hand scale) O₂ transfer in the presence of CO₂ exchange; (---------, left-hand scale) and (----- ---, right-hand scale) O₂ transfer in the absence of CO₂ exchange.
The magnitude of the Haldane effect on the oxygenation case is given in Figures 6.15 and 6.16 for the Type 1 and 2 membrane tubes, respectively; on the deoxygenation case, Figures 6.17 and 6.18. The left-hand plots of Figures 6.15 - 6.18 present the results of total CO₂ content change versus transit time; and the right-hand plots give the change in mixed mean $P_{CO_2}$ and intracellular hemoglobin carbamate concentration. In the absence of simultaneous O₂ transfer, the total CO₂ content changes calculated are less than than the changes that occur under the condition of collateral O₂/CO₂ exchange. The calculated total CO₂ content changes at $z=L$ are tabulated in Table 6.13. As shown in the table, according to this model $\approx 10\%$ of total CO₂ transferred is linked to simultaneous O₂ transfer for the oxygenation case; and $\approx 5\%$ for the deoxygenation case. However, it should be mentioned that the fraction of total CO₂ exchange that is accounted for by the Haldane effect should be larger than what is estimated here. The reason is that only half of the Haldane effect, the effect of O₂ carriage on carbamino compounds, is accounted for in this model. The additional bicarbonate contribution in the presence of simultaneous O₂ exchange which is due to the altered buffering capacity of hemoglobin is not included in this model. The increased acidity of oxyhemoglobin leads to an increase in the intracellular H⁺ and allows more intracellular HCO₃⁻ change. Plasma HCO₃⁻ would be affected by this effect because of the HCO₃⁻/Cl⁻ exchange. This altered buffer capacity of hemoglobin is not completely quantified; as a result, a mathematical description of this relationship is not included in this model.
Figure 6.15: Influence of the Haldane effect on rate of CO₂ elimination from human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]_{total,in}=22.4 mM). Curves: theoretical simulation of the situations; (———) CO₂ transfer in the presence of O₂ exchange, (-----) CO₂ transfer in the absence of O₂ exchange. The right panel gives both changes in mixed mean $P_{CO₂}$ and $[HbCO₂]_{rbc}$ as functions of transit time ($P_{CO₂,in}=52.5$ mmHg, $[HbCO₂]_{rbc,in}=5.08$ mM). Curves: theoretical simulation of the situations; (———, left-hand scale) and (———, right-hand scale) CO₂ transfer in the presence of O₂ exchange; (-----, left-hand scale) and (-----, right-hand scale) CO₂ transfer in the absence of O₂ exchange.
Figure 6.16: Influence of the Haldane effect on rate of CO₂ elimination from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]total,in=22.4 mM). Curves: theoretical simulation of the situations; (-----) CO₂ transfer in the presence of O₂ exchange, (-----) CO₂ transfer in the absence of O₂ exchange. The right panel gives both changes in mixed mean $P_{CO₂}$ and $[HbCO₂]_{rbc}$ as functions of transit time ($P_{CO₂,in}=52.5$ mmHg, $[HbCO₂]_{rbc,in}=5.08$ mM). Curves: theoretical simulation of the situations; (-----, left-hand scale) and (-----, right-hand scale) CO₂ transfer in the presence of O₂ exchange; (-----, left-hand scale) and (-----, right-hand scale) CO₂ transfer in the absence of O₂ exchange.
Figure 6.17: Influence of the Haldane effect on rate of CO₂ uptake by human whole blood flowing in a Type I artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time (---) CO₂ transfer in the presence of O₂ exchange, (...) CO₂ transfer in the absence of O₂ exchange. The right panel gives both changes in mixed mean PₐCO₂ and [HbCO₂]₀.Ó as functions of transit time (---) CO₂ transfer in the presence of O₂ exchange, (...) CO₂ transfer in the absence of O₂ exchange. (---, left-hand scale) and (...) right-hand scale) CO₂ transfer in the absence of O₂ exchange.
Figure 6.18: Influence of the Haldane effect on rate of CO₂ uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time [HCO₃⁻] for different cases: Case 1: P, CO₂; Case 2: [HCO₃⁻]. The right panel gives both changes in mixed mean P, CO₂ and [HCO₃⁻], as functions of transit time (P, CO₂ = 28.8 mmHg, [HCO₃⁻] = 1.31 mM). Curves: theoretical simulation of the situations: (- - - - -), CO₂ transfer in the absence of O₂ exchange; (--- --- ---), CO₂ transfer in the presence of O₂ exchange; (----- -----), CO₂ transfer in the absence of O₂ exchange.
Table 6.13: Influence of the Haldane effect on CO₂ transfer for both oxygenation and deoxygenation cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Δ[(CO₂)<em>{total}] = [(CO₂)</em>{total}(ε=L) - (CO₂)_{total}(ε=0)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ transport alone</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)†</td>
<td>-11.8 mM</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 μm) *</td>
<td>-11.6 mM</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>16.6 mM</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 μm) *</td>
<td>19.2 mM</td>
</tr>
</tbody>
</table>

† Type 1 membrane tube: Q=12.5 ml/hr.
* Type 2 membrane tube: Q=9 μl/hr.
† Values in parentheses denote the % increase in the amount of total CO₂ transfer which is calculated as: % ↑ = \( \frac{\Delta[(CO₂)_{total}(O₂/CO₂ \text{ transfer})] - \Delta[(CO₂)_{total}(CO₂ \text{ transfer alone})]}{\Delta[(CO₂)_{total}(O₂/CO₂ \text{ transfer})]} \).

6.2.2.b Effects of Extracellular Carbonic Anhydrase Activity and Buffering Capacity

CO₂ transport in the microvessels as functions of the transit time and extracellular carbonic anhydrase activity for oxygenation situation is shown in Figures 6.19 and 6.20 for Type 1 and Type 2 membrane tubes, respectively; for deoxygenation situation, Figures 6.21 and 6.22. On the left hand side of these figures, the computed change in the total CO₂ content is shown for three different values of the rates of the extracellular CO₂ hydration/dehydration reactions using \( \dot{A}_{pr} \) of 1, 100 and 6500; and on the right hand side, the computed changes in plasma HCO₃⁻ concentration and pH. \( \dot{A}_{pr}=1 \) and \( \beta_{pr}=5.5 \) mM H⁺/pH represent the case where no carbonic anhydrase activity nor any additional buffers are added to the plasma. As it is illustrated in these figures, CO₂ transfer increases with the enhancement of plasma carbonic anhydrase activity. The calculated total CO₂ content changes at the outlet of the membrane tubes are
Figure 6.19: Effect of extracellular catalysis of CO₂ hydration/dehydration reactions on rate of CO₂ elimination from human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]_{total,in} = 22.4 mM). Curves: theoretical simulation of the situations; (----) \( \hat{A}_{ptl}=1 \), (- - - - - -) \( \hat{A}_{ptl}=100 \), (- - - - - -) \( \hat{A}_{ptl}=6500 \) and \( \beta_{ptl}=5.5 \) mM H⁺/pH; (---) \( \hat{A}_{ptl}=6500 \) and \( \beta_{ptl}=54.6 \) mM H⁺/pH. The right panel gives both changes in mixed mean \([HCO₃]_{ptl} \) and \( pH_{ptl} \) as functions of transit time ([HCO₃]_{ptl,in} = 25.6 mM, \( pH_{ptl,in} = 7.28 \)). Curves: theoretical simulation of the situations; (----, left-hand scale) and (---, right-hand scale) \( \hat{A}_{ptl}=1 \) and \( \beta_{ptl}=5.5 \) mM H⁺/pH; (- - - - - - , left-hand scale) and (- - - - - - - - - - - , right-hand scale) \( \hat{A}_{ptl}=100 \) and \( \beta_{ptl}=5.5 \) mM H⁺/pH; (---, left-hand scale) and (---, right-hand scale) \( \hat{A}_{ptl}=6500 \) and \( \beta_{ptl}=5.5 \) mM H⁺/pH.
Figure 6.20: Effect of extracellular catalysis of CO₂ hydration/dehydration reactions on rate of CO₂ elimination from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]_{total,in}=22.4 mM). Curves: theoretical simulation of the situations; (——) \( \hat{A}_{pt}=1 \), (---- - - - -) \( \hat{A}_{pt}=100 \), (-----) \( \hat{A}_{pt}=6500 \) and \( \beta_{pt}=5.5 \) mM H⁺/pH; (-----) \( \hat{A}_{pt}=6500 \) and \( \beta_{pt}=54.6 \) mM H⁺/pH. The right panel gives both changes in mixed mean \([HCO₃^-]_{pl}\) and \(pH_{pl}\) as functions of transit time \([HCO₃^-]_{pl,in}=25.6 \) mM, \(pH_{pl,in}=7.28\). Curves: theoretical simulation of the situations; (—— — — , left-hand scale) and (----- — — , right-hand scale) \( \hat{A}_{pt}=1 \) and \( \beta_{pt}=5.5 \) mM H⁺/pH; (----- — — , left-hand scale) and (----- — — , right-hand scale) \( \hat{A}_{pt}=100 \) and \( \beta_{pt}=5.5 \) mM H⁺/pH; (----- — — , left-hand scale) and (----- — — , right-hand scale) \( \hat{A}_{pt}=6500 \) and \( \beta_{pt}=5.5 \) mM H⁺/pH.
Figure 6.21: Effect of extracellular catalysis of CO₂ hydration/dehydration reactions on rate of CO₂ removal by human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]_{total,in}=24.4 mM). Curves: theoretical simulation of the situations; (—) \( \hat{A}_{pl}=1 \), (— — — — — -) \( \hat{A}_{pl}=100 \), (— — — —) \( \hat{A}_{pl}=6500 \) and \( \beta_{pl}=5.5 \) mM H⁺/pH; (— — — —) \( \hat{A}_{pl}=6500 \) and \( \beta_{pl}=54.6 \) mM H⁺/pH. The right panel gives both changes in mixed mean \([HCO₃]_{pl}\) and \(pH_{pl}\) as functions of transit time ([HCO₃]_{pl,in}=31.2 mM, \(pH_{pl,in}=7.66\)). Curves: theoretical simulation of the situations; (—, left-hand scale) and (— —, right-hand scale) \( \hat{A}_{pl}=1 \) and \( \beta_{pl}=5.5 \) mM H⁺/pH; (— — — — , left-hand scale) and (— — — — — — — — , right-hand scale) \( \hat{A}_{pl}=100 \) and \( \beta_{pl}=5.5 \) mM H⁺/pH; (— — — — , left-hand scale) and (— — — — — , right-hand scale) \( \hat{A}_{pl}=6500 \) and \( \beta_{pl}=5.5 \) mM H⁺/pH.
Figure 6.22: Effect of extracellular catalysis of CO₂ hydration/dehydration reactions on rate of CO₂ removal by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time ([CO₂]_total, in=24.4 mM). Curves: theoretical simulation of the situations; (----) $\hat{A}_{pt}=1$, (-----) $\hat{A}_{pt}=100$, (--- - - -) $\hat{A}_{pt}=6500$ and $\beta_{pt}=5.5$ mM H⁺/pH; (----- ---) $\hat{A}_{pt}=6500$ and $\beta_{pt}=54.6$ mM H⁺/pH. The right panel gives both changes in mixed mean $[HCO₃⁻]_{pl}$ and $pH_{pl}$ as functions of transit time ($[HCO₃⁻]_{pl,in}=31.2$ mM, $pH_{pl,in}=7.66$). Curves: theoretical simulation of the situations; (---, left-hand scale) and (---, right-hand scale) $\hat{A}_{pt}=1$ and $\beta_{pt}=5.5$ mM H⁺/pH; (-----, left-hand scale) and (------, right-hand scale) $\hat{A}_{pt}=100$ and $\beta_{pt}=5.5$ mM H⁺/pH; (-----, left-hand scale) and (----- ---, right-hand scale) $\hat{A}_{pt}=6500$ and $\beta_{pt}=5.5$ mM H⁺/pH.
tabulated in Table 6.14. With catalysis similar to that present inside the RBC ($\Delta_p=6500$), the increment in CO$_2$ transfer represent a 12-43% increase for the oxygenation case and 8-13% increases for the deoxygenation case. It is worth noting that the maximum increment in CO$_2$ transfer for the conditions of $\Delta_p=6500$ and $\beta_p=5.5$ mM H$^+/pH$ (normal plasma buffering capacity) is less than the case where $\Delta_p=6500$ and $\beta_p=54.6$ mM H$^+/pH$ (buffering capacity equivalent to that inside the RBC). This result is due to the compartmentalization of blood buffers. Normal plasma buffer is only $\approx 10\%$ of that of RBC interior, and with rapid CO$_2$ dehydration (hydration) of plasma HCO$_3^-$ there is a concomitant reduction (increase) of H$^+$, which reduces the speed of the dehydration (hydration) reaction. Therefore, both plasma enzyme and buffering of pH changes are necessary to maintain rapid transfer.

It is interesting to observe that the model predicts a $|\Delta pH_p|$ of $\approx 0.05$ pH units at $z=L$ for 27-μm-diameter vessels which is much smaller in comparison to a $|\Delta pH_p|$ of $\approx 0.4$ pH units for 1.47-mm-diameter vessels. For illustration, consider the case of blood oxygenation accompanied by CO$_2$ removal. During the gas exchange CO$_2$ diffuses into the environmental gas space, and $P_{CO2}$ of blood decreases. Intracellular HCO$_3^-$ falls rapidly as it is converted to CO$_2$ in the presence of carbonic anhydrase. Plasma HCO$_3^-$ decreases much slowly because the chemical conversion is uncatalyzed, and most of the decrease in HCO$_3^-$ of the plasma is by way of the anion exchange. Within the RBCs, CO$_2$, HCO$_3^-$ and H$^+$ remain essentially in equilibrium; however, the uncatalyzed reaction occurs so slowly that the plasma H$^+$ concentration changes only slightly while CO$_2$ and HCO$_3^-$ decreases. The absence of carbonic anhydrase in plasma does not restrict CO$_2$ exchange; however, it does restrict the rate of plasma pH changes. For the large vessels with diameters of 1.47 mm, the residence time of the RBCs in the membrane tube ($\approx$ several minutes) is long compared to the time scale of the uncatalyzed extracellular CO$_2$ hydration/dehydration reactions (half time of $\approx 10$
Table 6.14

Effect of extracellular catalysis of CO₂ hydration/dehydration reactions ($\hat{A}_{pi}$) and extracellular buffering capacity ($\beta_{pi}$) on CO₂ transfer for both oxygenation (accompanied by CO₂ elimination) and deoxygenation (accompanied by CO₂ uptake) cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>$\Delta$[CO₂]<em>{total} = [CO₂]</em>{total}(z=L) - [CO₂]_{total}(z=0) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_{pi}$=5.5 mM H⁺/pH</td>
</tr>
<tr>
<td></td>
<td>$\hat{A}_{pi}=1$</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)†</td>
<td>-13.0 (39% ↑)</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 µm)*</td>
<td>-13.1 (5% ↑)</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>17.4 (9% ↑)</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 µm)*</td>
<td>20.6 (5% ↑)</td>
</tr>
</tbody>
</table>

† Type 1 membrane tube: $Q$=12.5 ml/hr.
* Type 2 membrane tube: $Q$=9 µl/hr.

† Values in parentheses denote the % increase in the amount of total CO₂ transfer which is calculated as:

$$\% \uparrow = \frac{\Delta [CO₂]_{local}(\hat{A}_{pi}, \beta_{pi}) - \Delta [CO₂]_{local}(\hat{A}_{pi}=1, \beta_{pi}=5.5 \text{ mM H}^+/\text{pH})}{\Delta [CO₂]_{local}(\hat{A}_{pi}=1, \beta_{pi}=5.5 \text{ mM H}^+/\text{pH})}.$$
Table 6.15

Effect of extracellular catalysis of CO₂ hydration/dehydration reactions (Åₚₚ) and extracellular buffering capacity (βₚₚ) on plasma pH changes for both oxygenation (accompanied by CO₂ elimination) and deoxygenation (accompanied by CO₂ uptake) cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>(\Delta pH_{pl} = pH_{pl}(z=L) - pH_{pl}(z=0))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta_{pl}=5.5\text{ mM H}^+/{\text{pH}})</td>
</tr>
<tr>
<td></td>
<td>(\hat{\lambda}_{pl}=1)</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)†</td>
<td>0.433 (20% ↑) (\frac{\Delta pH_{pl}(\hat{\lambda}<em>{pl}, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}})}{\hat{\lambda}<em>{pl}=1, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}}}))</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 μm)*</td>
<td>0.023 (15-fold ↑) (\frac{\Delta pH_{pl}(\hat{\lambda}<em>{pl}=1, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}})}{\hat{\lambda}<em>{pl}=6500, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}}}))</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>- 0.386 (21% ↑) (\frac{\Delta pH_{pl}(\hat{\lambda}<em>{pl}=1, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}})}{\hat{\lambda}<em>{pl}=6500, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}}}))</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 μm)*</td>
<td>- 0.050 (7-fold ↑) (\frac{\Delta pH_{pl}(\hat{\lambda}<em>{pl}=1, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}})}{\hat{\lambda}<em>{pl}=6500, \beta</em>{pl}=5.5\text{ mM H}^+/{\text{pH}}}))</td>
</tr>
</tbody>
</table>

† Type 1 membrane tube: \(Q=12.5\text{ ml/hr}\).

* Type 2 membrane tube: \(Q=9\text{ μl/hr}\).

‡ Values in parentheses denote the increase in the change of plasma pH which is calculated as:

\((\% \uparrow)\) or (fold \(\uparrow\)) = \(\frac{\Delta pH_{pl}(\hat{\lambda}_{pl}, \beta_{pl}=5.5\text{ mM H}^+/{\text{pH}}) - \Delta pH_{pl}(\hat{\lambda}_{pl}=1, \beta_{pl}=5.5\text{ mM H}^+/{\text{pH}})}{\Delta pH_{pl}(\hat{\lambda}_{pl}=1, \beta_{pl}=5.5\text{ mM H}^+/{\text{pH}})}\).

# Values in parentheses denote % the decrease in the change of plasma pH which is calculated as:

\(\% \downarrow = \frac{\Delta pH_{pl}(\hat{\lambda}_{pl}=6500, \beta_{pl}=5.5\text{ mM H}^+/{\text{pH}}) - \Delta pH_{pl}(\hat{\lambda}_{pl}=6500, \beta_{pl}=54.6\text{ mM H}^+/{\text{pH}})}{\Delta pH_{pl}(\hat{\lambda}_{pl}=6500, \beta_{pl}=5.5\text{ mM H}^+/{\text{pH}})}\).
sec, Klocke (1987)); consequently, plasma pH continues to change as blood travels down the vessels. For the smaller tubes with diameters of 27 μm, the residence time of the RBCs is of the order of one sec; as a result, plasma pH remains essentially unchanged. However, in the cases where additional carbonic anhydrase is added to the plasma of a 27-μm-diameter vessel, the magnitude of ΔpH_{pl} undergoes a 10 - 20 fold increase (Table 6.15).

6.2.2.c Effects of Red Blood Cell HCO_3^-/Cl^- Exchange Kinetics

Another major determinant of CO_2 transport in blood involves the kinetics of HCO_3^-/Cl^- exchange across the RBC membrane. Since under normal physiological conditions, ~80% of CO_2 transfer is derived from the hydration of intra- and extracellular HCO_3^-. The rate of anion exchange is important. The impact of the anion exchange on CO_2 transfer is studied here by varying T_{tot}; results are shown for T_{tot} ranging from 0.02x10^6 to 1x10^7. T_{tot}=1x10^6 represents normal RBC membrane; T_{tot}=0.02x10^6 and 0.5x10^6 represent abnormal or drug-inhibited RBC membrane; T_{tot}=1x10^7 represents a 10-fold increase in anion transporters per RBC. The influence of the anion exchange on CO_2 transport is shown in Figures 6.23 and 6.24 for the oxygenation case in the 27-μm-diameter tube; in Figures 6.25 and 6.26 for the deoxygenation case. On the left hand side of Figures 6.23 and 6.25 the computed total CO_2 content and change is given as a function of the transit time and T_{tot}; and on the right hand side, the changes in both the intra- and extracellular HCO_3^-. Figures 6.24 and 6.26 present effect of anion exchange on pH regulation and Cl^- concentration change; the left-hand plots show changes in both intra- and extracellular pH versus transit time; the right-hand plots, changes in both intra- and extracellular Cl^- . These results illustrate that inhibition of RBC anion exchange affects CO_2 transport and pH changes (also see Tables 6.16 and 6.17). Because some commonly used drugs and anesthetics are known to inhibit the RBC anion exchange, the results may have
Figure 6.23: Effect of RBC HCO$_3^-$/Cl$^-$ exchange on rate of CO$_2$ elimination from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO$_2$ content change as a function of transit time ([CO$_2$]$_{total,in}$=22.4 mM). Curves: theoretical simulation of the situations; (-----) $T_{tot}$=0.02x10$^6$, (- - - -) $T_{tot}$=0.5x10$^6$, (- - - - - -) $T_{tot}$=1x10$^6$ and (-----) $T_{tot}$=1x10$^7$. The right panel gives both changes in mixed mean intra- and extracellular HCO$_3^-$ concentrations as functions of transit time ([HCO$_3^-$]$_{rbc,in}$=9.42 mM, [HCO$_3^-$]$_{pl,in}$=25.6 mM). Curves: theoretical simulation of the situations; (-----, left-hand scale) and (-----, right-hand scale) $T_{tot}$=0.02x10$^6$; (- - - - , left-hand scale) and (----- , right-hand scale) $T_{tot}$=0.5x10$^6$; (-----, left-hand scale) and (----- , right-hand scale) $T_{tot}$=1x10$^6$. 
Figure 6.24: Effect of RBC anion exchange on rate of CO₂ elimination from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives both the changes in mixed mean intra- and extracellular pH as functions of transit time \( (pH_{\text{rbc, in}}=7.06, pH_{\text{pl, in}}=7.28) \). Curves: theoretical simulation of the situations; (———, left-hand scale) and (— — —, right-hand scale) \( T_{\text{tot}}=0.02\times10^6 \); ( — — —, left-hand scale) and ( — — —, right-hand scale) \( T_{\text{tot}}=0.5\times10^6 \); ( — — —, left-hand scale) and ( — — —, right-hand scale) \( T_{\text{tot}}=1\times10^6 \). The right panel gives both changes in mixed mean intra- and extracellular Cl⁻ concentrations as functions of transit time \( ([Cl^-]_{\text{rbc, in}}=36.7 \text{ mM}, [Cl^-]_{\text{pl, in}}=98.2 \text{ mM}) \). Curves: theoretical simulation of the situations; (———, left-hand scale) and (— — —, right-hand scale) \( T_{\text{tot}}=0.02\times10^6 \); ( — — —, left-hand scale) and ( — — —, right-hand scale) \( T_{\text{tot}}=0.5\times10^6 \); ( — — —, left-hand scale) and ( — — —, right-hand scale) \( T_{\text{tot}}=1\times10^6 \).
Figure 6.25: Effect of RBC HCO$_3^-$/Cl$^-$ exchange on rate of CO$_2$ uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO$_2$ content change as a function of transit time ($[CO_2]_{total,in}=24.4$ mM). Curves: theoretical simulation of the situations; (-----) $T_{tot}=0.02 \times 10^6$, (------) $T_{tot}=0.5 \times 10^6$, (---------) $T_{tot}=1 \times 10^6$ and (--- ---) $T_{tot}=1 \times 10^7$. The right panel gives both changes in mixed mean intra- and extracellular HCO$_3^-$ concentrations as functions of transit time ($[HCO_3^-]_{rbc,in}=12.0$ mM, $[HCO_3^-]_{pl,in}=31.2$ mM). Curves: theoretical simulation of the situations; (-----, left-hand scale) and (----, right-hand scale) $T_{tot}=0.02 \times 10^6$; (------, left-hand scale) and (-----, right-hand scale) $T_{tot}=0.5 \times 10^6$; (---, left-hand scale) and (--- ---, right-hand scale) $T_{tot}=1 \times 10^6$. 

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Figure 6.26: Effect of RBC anion exchange on rate of CO₂ uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives both the changes in mixed mean intra- and extracellular pH as functions of transit time (pH_rbc, in=7.44, pH_pl, in=7.66). Curves: theoretical simulation of the situations; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=0.02 \times 10^6$; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=0.5 \times 10^6$; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=1 \times 10^6$. The right panel gives both changes in mixed mean intra- and extracellular Cl⁻ concentrations as functions of transit time ([Cl⁻] rbc, in=40.2 mM, [Cl⁻] pl, in=107.8 mM). Curves: theoretical simulation of the situations; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=0.02 \times 10^6$; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=0.5 \times 10^6$; (— — —, left-hand scale) and (— — —, right-hand scale) $T_{tot}=1 \times 10^6$. 
Table 6.16

Effect of anion exchange on CO₂ transfer for both oxygenation (accompanied by CO₂ elimination) and deoxygenation (accompanied by CO₂ uptake) cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>$\Delta [CO₂]<em>{total} = [CO₂]</em>{total}(z=L) - [CO₂]_{total}(z=0)$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{tot}=0.02\times10^6$</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 µm)‡</td>
<td>-8.0 (39% ↓)*</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 µm)‡</td>
<td>18.1 (12% ↓)</td>
</tr>
</tbody>
</table>

‡ Type 2 membrane tube: $Q=9$ µl/hr.

* Values in parentheses denote the % decrease in the amount of total CO₂ transfer which is calculated as:

$$\% \downarrow = \frac{\Delta [CO₂]_{total}(T_{tot}=1\times10^6) - \Delta [CO₂]_{total}(T_{tot})}{\Delta [CO₂]_{total}(T_{tot}=1\times10^6)}.$$

¶ Values in parentheses denote the % increase in the amount of total CO₂ transfer which is calculated as:

$$\% \uparrow = \frac{\Delta [CO₂]_{total}(T_{tot}) - \Delta [CO₂]_{total}(T_{tot}=1\times10^6)}{\Delta [CO₂]_{total}(T_{tot}=1\times10^6)}.$$
Table 6.17

Effect of anion exchange on both intra- and extracellular \( p\text{H} \) changes for both oxygenation (accompanied by CO\(_2\) elimination) and deoxygenation (accompanied by CO\(_2\) uptake) cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>( \Delta pH_{rbc} = pH_{rbc}(z=L) - pH_{rbc}(z=0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_{tot}=0.02\times10^6 )</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 ( \mu \text{m} ))( ^\dagger )</td>
<td>0.016 (91% ( \downarrow ))</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 ( \mu \text{m} ))( ^\dagger )</td>
<td>-0.012 (92% ( \downarrow ))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cases</th>
<th>( \Delta pH_{pl} = pH_{pl}(z=L) - pH_{pl}(z=0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_{tot}=0.02\times10^6 )</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 ( \mu \text{m} ))( ^\dagger )</td>
<td>0.043 (84% ( \uparrow ))( ^\dagger )</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 ( \mu \text{m} ))( ^\dagger )</td>
<td>-0.064 (28% ( \uparrow ))</td>
</tr>
</tbody>
</table>

\( ^\dagger \) Type 2 membrane tube: \( Q=9 \mu \text{l/hr} \).

* Values in parentheses denote the % decrease in the change of RBC \( p\text{H} \) which is calculated as:

\[
% \downarrow = \frac{\Delta pH_{rbc}(T_{tot}=1\times10^6) - \Delta pH_{rbc}(T_{tot})}{\Delta pH_{rbc}(T_{tot}=1\times10^6)}\, .
\]

\( ^\dagger \) Values in parentheses denote the % increase in the change of plasma \( p\text{H} \) which is calculated as:

\[
% \uparrow = \frac{\Delta pH_{pl}(T_{tot}) - \Delta pH_{pl}(T_{tot}=1\times10^6)}{\Delta pH_{pl}(T_{tot}=1\times10^6)}\, .
\]
important clinical implications on blood CO₂ transport and pH regulation in small microvessels. Due to the fact that a smaller HCO₃⁻/Cl⁻ flux is involved for a 1.47-mm-diameter tube (about 2-3 order of magnitudes smaller than that observed for a 27-μm-diameter tube), changes in the quantity $T_{tot}$ only introduced small changes in CO₂ transport. Those changes, which amount to less than 5% of the total transport, are of minor importance; consequently, those results are not shown.

For illustration, let’s consider blood oxygenation accompanied by CO₂ removal in the case where 98% of the HCO₃⁻/Cl⁻ exchange is inhibited (case 1 in Figure 6.23). The decrease in the efficiency of anion transport results in a ≈ 39% fall in CO₂ elimination (Table 6.16). In this situation, the accessibility of intracellular carbonic anhydrase activity to the extracellular HCO₃⁻ is further limited. As a result, CO₂ normally eliminated from blood that had been in the form of plasma HCO₃⁻ when the blood entered the microvessel is not available to be excreted. The left-hand plot of Figure 6.23 depicts a lowest $\Delta[HCO₃⁻]_{pl}$ and highest $\Delta[HCO₃⁻]_{rbc}$ for case 1 where 98% of the anion transport capability is impaired. Although there is an increase in intracellular HCO₃⁻ removal from blood, the magnitude of this additional $\Delta[HCO₃⁻]_{rbc}$ is not sufficient to compensate for the decrease in the elimination of plasma HCO₃⁻. The reason is that under the “normal” conditions of this computation ≈ 52% of the CO₂ elimination comes from the extracellular HCO₃⁻ pool, and ≈ 23% comes from the intracellular HCO₃⁻. In the case of the impeded anion exchange ($T_{tot}$=0.02x10⁶), only ≈ 13% of the CO₂ removal is derived from plasma HCO₃⁻, and ≈ 48% is derived from red cell HCO₃⁻.

In the case where the efficiency of the anion transporter is artificially increased by imposing a 10-fold increase in the total number of anion transporters per RBC, the total amount of CO₂ removal is increased by up to 9% (see Figure 6.23 and Table 6.16). This is due to the fact that during normal operation of the band 3 protein the
plasma HCO$_3^-$ pool is not depleted as much as it theoretically could be due to limitation of the quantity of bicarbonate ions that flow into the RBC to be hydrated. As the capacity of band 3 protein increases this limitation is decreased and blood increasingly behaves as though it had a common HCO$_3^-$ pool (or common carbonic anhydrase pool). In this situation, 56% of the $\Delta[CO_2]_{total}$ is attributed to $\Delta[HCO_3^-]_{pl}$; and 21%, $\Delta[HCO_3^-]_{rbc}$. It is also worth noting that for the blood deoxygenation accompanied by CO$_2$ uptake cases, $\Delta[CO_2]_{total}$ is affected to a lesser extent by the impairment of the HCO$_3^-$/Cl$^-$ exchange. The explanation for this observation is that during the reverse process of CO$_2$ uptake, intracellular carbonic anhydrase activity is accessible to the incoming bicarbonate source (in the form of CO$_2$) so that intracellular CO$_2$ hydration/dehydration reactions continue to occur and HCO$_3^-$ are mostly stored inside the RBCs. Although the extracellular HCO$_3^-$ carriage capacity is not fully utilized in this case, this magnitude of increase in $\Delta[HCO_3^-]_{rbc}$ is almost sufficient to compensate for the decrease in the $\Delta[HCO_3^-]_{pl}$. For the “normal” deoxygenation case considered here, 42% of the $\Delta[CO_2]_{total}$ is attributed to $\Delta[HCO_3^-]_{pl}$ and 41% is attributed to $\Delta[HCO_3^-]_{rbc}$. For abnormal or drug-inhibited case ($T_{tot}=0.02\times10^6$), only 5% of the $\Delta[CO_2]_{total}$ is derived from $\Delta[HCO_3^-]_{pl}$ and 75% from $\Delta[HCO_3^-]_{rbc}$.

6.2.2.d Effects of Hematocrit on Gas Transfer

The model is also used to examine rates of O$_2$ and CO$_2$ transfer in microvessels at different levels of discharge hematocrit; the range of hematocrit studied is 20-60%. For the oxygenation situation, O$_2$ uptake as functions of transit time are given in Figures 6.27 and 6.29 for the Types 1 and 2 artificial membrane tubes, respectively; and CO$_2$ elimination, Figures 6.28 and 6.30. For the deoxygenation situation, O$_2$ release results for the Types 1 and 2 artificial membrane tubes are given in Figures 6.31 and 6.33; and CO$_2$ uptake, Figures 6.32 and 6.34. The right-hand plots of Figures 6.27, 6.29, 6.31 and 6.33 show the computed changes in $[O_2]_{total}$; the left-hand plots of
Figure 6.27: Effect of discharge hematocrit on rate of O\(_2\) uptake by human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total O\(_2\) content change as a function of transit time. Curves: theoretical simulation of the situations; (———) \(H_D=0.20\) and \([O_2]_{total,in}=0.53\) mM, (-----) \(H_D=0.42\) and \([O_2]_{total,in}=1.09\) mM, (— - —) \(H_D=0.60\) and \([O_2]_{total,in}=1.55\) mM. The right panel gives both changes in mixed mean \(P_{O2}\) and \(O_2\) saturation as functions of transit time (\(P_{O2,in}=14.0\) mmHg, \(S_{in}=0.12\)). Curves: theoretical simulation of the situations; (———, left-hand scale) and (— — , right-hand scale) \(H_D=0.20\); (-----, left-hand scale) and (-----, right-hand scale) \(H_D=0.42\); (— — , left-hand scale) and (———, right-hand scale) \(H_D=0.60\).
Figure 6.28: Effect of discharge hematocrit on rate of CO₂ elimination from human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time. Curves: theoretical simulation of the situations; (———-) \( H_D = 0.20 \) and \( [CO_2]_{total, in} = 24.9 \) mM, (-----) \( H_D = 0.42 \) and \( [CO_2]_{total, in} = 22.4 \) mM, (----) \( H_D = 0.60 \) and \( [CO_2]_{total, in} = 20.4 \) mM. The right panel gives both changes in mixed mean \( P_{CO_2} \) and \( [HCO_3^-]_{total} \) as functions of transit time (\( P_{CO_2, in} = 52.5 \) mmHg). Curves: theoretical simulation of the situations; (——, left-hand scale) and (——, right-hand scale) \( H_D = 0.20 \) and \( [HCO_3^-]_{total, in} = 22.3 \) mM; (-----, left-hand scale) and (-----, right-hand scale) \( H_D = 0.42 \) and \( [HCO_3^-]_{total, in} = 18.8 \) mM; (———, left-hand scale) and (———, right-hand scale) \( H_D = 0.60 \) and \( [HCO_3^-]_{total, in} = 16.0 \) mM.
Figure 6.29: Effect of discharge hematocrit on rate of O$_2$ uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total O$_2$ content change as a function of transit time. Curves: theoretical simulation of the situations; (— — —) $H_D=0.20$ and [$O_2$]$_{total,in}=0.53$ mM, (— — — —) $H_D=0.42$ and [$O_2$]$_{total,in}=1.09$ mM, (— — —) $H_D=0.60$ and [$O_2$]$_{total,in}=1.55$ mM. The right panel gives both changes in mixed mean $P_{O2}$ and O$_2$ saturation as functions of transit time ($P_{O2,in}=14.0$ mmHg, $S_{in}=0.12$). Curves: theoretical simulation of the situations; (— — —, left-hand scale) and (— —, right-hand scale) $H_D=0.20$; (— — — —, left-hand scale) and (— — — —, right-hand scale) $H_D=0.42$; (— — — —, left-hand scale) and (— — — —, right-hand scale) $H_D=0.60$. 
Figure 6.30: Effect of discharge hematocrit on rate of CO₂ elimination from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time. Curves: theoretical simulation of the situations; (———) $H_D=0.20$ and $[CO_2]_{\text{total, in}}=24.9$ mM, (-----) $H_D=0.42$ and $[CO_2]_{\text{total, in}}=22.4$ mM, (---) $H_D=0.60$ and $[CO_2]_{\text{total, in}}=20.4$ mM. The right panel gives both changes in mixed mean $P_{CO_2}$ and $[HCO_3^-]_{\text{total}}$ as functions of transit time ($P_{CO_2,\text{in}}=52.5$ mmHg). Curves: theoretical simulation of the situations; (———, left-hand scale) and (——, right-hand scale) $H_D=0.20$ and $[HCO_3^-]_{\text{total, in}}=22.3$ mM; (-----, left-hand scale) and (-----, right-hand scale) $H_D=0.42$ and $[HCO_3^-]_{\text{total, in}}=18.8$ mM; (——, left-hand scale) and (——, right-hand scale) $H_D=0.60$ and $[HCO_3^-]_{\text{total, in}}=16.0$ mM.
Figure 6.31: Effect of discharge hematocrit on rate of \( \text{O}_2 \) release from human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total \( \text{O}_2 \) content change as a function of transit time. Curves: theoretical simulation of the situations; (———) \( H_D=0.20 \) and \( [\text{O}_2]_{\text{total, in}}=4.09 \text{ mM} \), (-----) \( H_D=0.42 \) and \( [\text{O}_2]_{\text{total, in}}=8.43 \text{ mM} \), (--- ---) \( H_D=0.60 \) and \( [\text{O}_2]_{\text{total, in}}=12.1 \text{ mM} \). The right panel gives both changes in mixed mean \( P_{\text{O}_2} \) and \( \text{O}_2 \) saturation as functions of transit time (\( P_{\text{O}_2, in}=68.1 \text{ mmHg} \), \( S_{in}=0.94 \)). Curves: theoretical simulation of the situations; (———, left-hand scale) and (— — —, right-hand scale) \( H_D=0.20 \); (----- ——, left-hand scale) and (----- ———, right-hand scale) \( H_D=0.42 \); (——— ———, left-hand scale) and (— ——— ———, right-hand scale) \( H_D=0.60 \).
Figure 6.32: Effect of discharge hematocrit on rate of CO₂ uptake by human whole blood flowing in a Type 1 artificial membrane tube (see Table 6.11). The left panel gives the total CO₂ content change as a function of transit time. Curves: theoretical simulation of the situations; (———) $H_D=0.20$ and $[CO_2]_{total,in}=28.4$ mM, (---) $H_D=0.42$ and $[CO_2]_{total,in}=24.4$ mM, (-----) $H_D=0.60$ and $[CO_2]_{total,in}=21.3$ mM. The right panel gives both changes in mixed mean $P_{CO_2}$ and $[HCO_3^-]_{total}$ as functions of transit time ($P_{CO_2,in}=28.8$ mmHg). Curves: theoretical simulation of the situations; (——, left-hand scale) and (— —, right-hand scale) $H_D=0.20$ and $[HCO_3^-]_{total,in}=27.4$ mM; (-----, left-hand scale) and (· · · · ·, right-hand scale) $H_D=0.42$ and $[HCO_3^-]_{total,in}=23.1$ mM; (———, left-hand scale) and (— — —, right-hand scale) $H_D=0.60$ and $[HCO_3^-]_{total,in}=19.7$ mM.
Figure 6.33: Effect of discharge hematocrit on rate of O₂ release from human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total O₂ content change as a function of transit time. Curves: theoretical simulation of the situations; (—) $H_D=0.20$ and $[O_2]_{tot, in}=4.09$ mM, (——) $H_D=0.30$ and $[O_2]_{tot, in}=4.09$ mM. The right panel gives both changes in mixed mean $P_{O_2}$ and $O_2$ saturation as functions of transit time ($P_{O_2, in}=88.1$ mmHg, $S_a=0.94$). Curves: theoretical simulation of the situations; (—), left-hand scale and (——), right-hand scale; $H_D=0.20$, (——), left-hand scale and (——), right-hand scale; $H_D=0.60$. 
**Figure 6.34:** Effect of discharge hematocrit on rate of \( CO_2 \) uptake by human whole blood flowing in a Type 2 artificial membrane tube (see Table 6.11). The left panel gives the total \( CO_2 \) content change as a function of transit time. Curves: theoretical simulation of the situations; (- - - -) \( H_D = 0.20 \) and \( [CO_2]_{total,in} = 28.4 \) mM, ( - - - -) \( H_D = 0.42 \) and \( [CO_2]_{total,in} = 24.4 \) mM, ( - - - -) \( H_D = 0.60 \) and \( [CO_2]_{total,in} = 21.3 \) mM. The right panel gives both changes in mixed mean \( P_{CO_2} \) and \( [HCO_3^-]_{total} \) as functions of transit time (\( P_{CO_2,in} = 28.8 \) mmHg). Curves: theoretical simulation of the situations; (- - - , left-hand scale) and ( - - - , right-hand scale) \( H_D = 0.20 \) and \( [HCO_3^-]_{total,in} = 27.4 \) mM; (- - - - , left-hand scale) and ( - - - - , right-hand scale) \( H_D = 0.42 \) and \( [HCO_3^-]_{total,in} = 23.1 \) mM; ( - - - - , left-hand scale) and ( - - - - , right-hand scale) \( H_D = 0.60 \) and \( [HCO_3^-]_{total,in} = 19.7 \) mM.
those figures display the computed change in $P_{O_2}$ and $S$. The right-hand plots of Figures 6.28, 6.30, 6.32 and 6.34 present the computed changes in $[CO_2]_{total}$; the left-hand plots of those figures give the computed change in $P_{CO_2}$ and $[HCO_3^-]_{total}$. These results demonstrate that under the conditions of the computations both $\Delta[O_2]_{total}$ and $\Delta[CO_2]_{total}$ are affected by the discharge hematocrit of blood perfusing the microvessel.

The suspension with lower discharge hematocrit shows a larger change in $O_2$ saturation; this does not mean better $O_2$ transport properties. The diluted blood is easier to uptake or release to a given saturation level, because its total transport capacity is smaller. However, after any length $z$, the suspension with higher hematocrit carries or releases the greater amount of oxygen. The suspension with lower discharge hematocrit also gives a larger change in $P_{CO_2}$; this is due to the fact that $\alpha_{CO_2,pl}$ is greater than $\alpha_{CO_2,rbc}$. These results indicate that a reduction in $H_D$ from 42\% to 20\% leads to a $= 21\%-46\%$ reduction in $\Delta[O_2]_{total}$ compared with $= 16\%-38\%$ reduction in $\Delta[CO_2]_{total}$ (Table 6.18). An increase in $H_D$ from 42\% to 60\% results in $= 6\%-19\%$ increase in $\Delta[O_2]_{total}$ accompanied by $= 7\%-25\%$ increase in $\Delta[CO_2]_{total}$ (Table 6.18). The decrement in $\Delta[O_2]_{total}$ with reductions in $H_D$ is caused by a decrease in the $O_2$ carrying capacity of blood. Concomitant reductions in $\Delta[CO_2]_{total}$ with reductions of $H_D$ are caused mainly due to the decrease in the size of the high buffer capacity intraerythrocyte pool and diminished flux of $HCO_3^-/Cl^-$ across to the RBC membrane.
Table 6.18

Effect of discharge hematocrit ($H_D$) on $O_2$ and $CO_2$ transfer for both oxygenation (accompanied by $CO_2$ elimination) and deoxygenation (accompanied by $CO_2$ uptake) cases in artificial membrane tubes.

<table>
<thead>
<tr>
<th>Cases</th>
<th>$\Delta[O_2]<em>{\text{total}} = [O_2]</em>{\text{total}}(z=L) - [O_2]_{\text{total}}(z=0)$ (mM)</th>
<th>$\Delta[CO_2]<em>{\text{total}} = [CO_2]</em>{\text{total}}(z=L) - [CO_2]_{\text{total}}(z=0)$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H_D=0.20$</td>
<td>$H_D=0.42$</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)†</td>
<td>4.45 (46% ↓)</td>
<td>8.23</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 µm)*</td>
<td>3.77 (42% ↓)</td>
<td>6.45</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>- 1.28 (21% ↓)</td>
<td>- 1.62</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 µm)*</td>
<td>- 2.17 (27% ↓)</td>
<td>- 2.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cases</th>
<th>$\Delta[CO_2]<em>{\text{total}} = [CO_2]</em>{\text{total}}(z=L) - [CO_2]_{\text{total}}(z=0)$ (mM)</th>
<th>$\Delta[CO_2]<em>{\text{total}} = [CO_2]</em>{\text{total}}(z=L) - [CO_2]_{\text{total}}(z=0)$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H_D=0.20$</td>
<td>$H_D=0.42$</td>
</tr>
<tr>
<td>Oxygenation (I.D.=1.47 mm)†</td>
<td>- 10.9 (16% ↓)</td>
<td>- 13.0</td>
</tr>
<tr>
<td>Oxygenation (I.D.=27 µm)*</td>
<td>- 9.0 (31% ↓)</td>
<td>- 13.1</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=1.47 mm)†</td>
<td>12.7 (32% ↓)</td>
<td>17.4</td>
</tr>
<tr>
<td>Deoxygenation (I.D.=27 µm)*</td>
<td>12.7 (38% ↓)</td>
<td>20.6</td>
</tr>
</tbody>
</table>

† Type 1 membrane tube: $Q=12.5$ ml/hr.

* Type 2 membrane tube: $Q=9$ µl/hr.

† Values in parentheses denote the % decrease in the amount of total $O_2$ or $CO_2$ transfer which is calculated as:

$$\% \downarrow = \frac{\Delta [C_i]_{\text{total}}(H_D=0.42) - \Delta [C_i]_{\text{total}}(H_D=0.20)}{\Delta [C_i]_{\text{total}}(H_D=0.42)}$$

# Values in parentheses denote the % increase in the amount of total $O_2$ or $CO_2$ transfer which is calculated as:

$$\% \uparrow = \frac{\Delta [C_i]_{\text{total}}(H_D=0.60) - \Delta [C_i]_{\text{total}}(H_D=0.42)}{\Delta [C_i]_{\text{total}}(H_D=0.42)}$$
6.3 Summary

The blood-gas chemistry has been of interest to numerous investigators (see Chapters 2 and 3). Many investigators made enormous contributions to our understanding of this intricate physicochemical system. In this study, the findings of these other investigators including the simultaneous interactions of hemoglobin with O₂, CO₂, H⁺ and DPG, CO₂ hydration/dehydration reactions and the buffering action of various buffer systems are incorporated into a mathematical model of gas transport and pH regulation by blood flowing in microvessels. There are a number of refinements in the present analysis compared to previous attempts at a quantitative examination of the chemical and transport processes by blood flowing in microvessels. The transport model developed here takes the particulate, or two-phase, nature of blood into account, while previous analysis assumed blood to be a continuum. This discrete model not only considers the fact that most of the O₂ carried by blood is bound to hemoglobin which is encapsulated inside the RBCs, but also takes into account the compartmentalization of carbonic anhydrase activity and buffers. This analysis also includes the cooperative interactions of various components in the blood, while most of prior studies neglect the Bohr and Haldane effects. Ionic movements (HCO₃⁻ and Cl⁻) across the RBC membrane are described in this work via a simplified ping-pong model, while previous models either did not include this exchange process or otherwise used a phenomenological passive diffusion approach. This model allows us to quantitate the effects of the many simultaneous processes involved in the gas exchange and gain insights not possible using previous approaches.

This discrete model of blood gas transport and pH regulation in microvessels is entirely predictive in the sense that there is no fitting to the O₂/CO₂ transport data. All the parameters are either physical properties or determined by ways independent of the
blood gas exchange experiments. Theoretical calculations simulating Voorhees' experiments show excellent agreement between the measured and predicted changes in $[O_2]_{total}$ and $[CO_2]_{total}$ (Voorhees, 1976) over a range of flowrates, hematocrits and inlet compositions (including $P_{O_2}, P_{CO_2}$ and $pH$). The computed results of $O_2$ transport generated by the continuum model also are in good agreement with the $O_2$ uptake data obtained for blood oxygenation accompanied by simultaneous $CO_2$ elimination experiments. However, the continuum model underpredicts the amount of $O_2$ release for the reverse process of blood deoxygenation accompanied with $CO_2$ uptake. This is due to the fact that the Bohr effect which plays a more important role during deoxygenation is neglected in the continuum model. In addition, the continuum approach yields inaccurate predictions of $CO_2$ transport. The overprediction of $CO_2$ transport by the continuum model is attributed to the local chemical equilibrium assumption. Although this assumption is applicable for describing $O_2$ transport in large vessels, it is determined here not to be appropriate for describing $CO_2$ transport due to the neglect of the compartmentalization of the carbonic anhydrase activity in blood.

On the basis of the results illustrated in Section 6.2.2.a, it is concluded that the Bohr effect is not of primary importance for blood oxygenation in the presence of simultaneous $CO_2$ elimination in microvessels (on the order of 10%). However, the Bohr effect is considerably more important for the deoxygenation process accompanied by simultaneous $CO_2$ uptake by blood (on the order of 20%). The computations also indicate that the Haldane effect (on the order of 10%) is only of secondary importance in $CO_2$ transfer in microvessels. However, it can potentially become more important upon incorporation of the altered buffering capacity of hemoglobin into the model.

As illustrated in Section 6.2.2.b, an enhancement in $CO_2$ transfer is observed when carbonic anhydrase activity is available to plasma. However, even in the
presence of large amounts of carbonic anhydrase activity available to plasma, CO₂ elimination and uptake by blood do not undergo a maximum increment. This is due to the fact that the magnitude of extracellular CO₂ hydration/dehydration reactions under these circumstances is limited by the buffer capacity of plasma which is only about 1/10 of that of the hemoglobin-rich RBC interior. Although the fractional changes in total CO₂ exchange are only moderately affected by the extent of catalysis of plasma CO₂ hydration/dehydration reactions in the 27-μm-diameter vessels (on the order of 10%), addition of carbonic anhydrase (Aₜₚₖ=6500) increases the magnitude of ΔpHₚₖ by over 10 fold.

The relationship between CO₂ transfer and HCO₃⁻/Cl⁻ exchange discussed in Section 6.2.2.c demonstrates that a decrease (increase) in total CO₂ transport is linked to a reduced (enhanced) capacity of the anion transporter. The fall in the magnitude of Δ[CO₂]ₜₚₖ is 39% when the capacity of the anion exchange is reduced by 98% for 27-μm-diameter vessels. It is expected that a reduction of the capacity by as little as 50% may be important in small capillaries where higher fluxes of CO₂ and O₂ are involved. Δ[CO₂]ₜₚₖ is substantially affected by the inhibition of band 3 protein, and the magnitudes of ΔpHₘₚₖ and ΔpHₚₖ are altered to a much greater extent. Even in the cases where Δ[CO₂]ₜₚₖ is only moderately affected, the magnitudes of ΔpHₘₚₖ and ΔpHₚₖ are still altered significantly. Because a number of commonly used drugs are known to inhibit anion exchanges across the human RBC membrane, these observations may have important clinical implications with respect to abnormal gas exchange and disturbed acid/base regulation due to impaired HCO₃⁻/Cl⁻ exchange.

The results presented in Section 6.2.2.d show that under the conditions of our computations both O₂ and CO₂ exchange are influenced by the discharge hematocrit of blood perfusing the vessels. The dependence of Δ[O₂]ₜₚₖ of O₂ transport on cell mass is well established, and these computations show the concomitant Δ[CO₂]ₜₚₖ. Any
decrement in $H_D$ (as with anemia) can, besides reducing $\Delta[O_2]_{total}$, also significantly diminish $\Delta[CO_2]_{total}$. The reduction in $\Delta[CO_2]_{total}$ is due to the decrease in the size of the high buffer capacity intracellular pool and decrease in the total number of anion transporters available for $\text{HCO}_3^-/\text{Cl}^-$ exchange.

Computations of gas exchange and $pH$ regulation are limited by the model chosen and the data used to characterize the model parameters. Previous attempts to describe this transport system have involved using continuum approximations and lumped-parameter approaches. As a result, effective properties and phenomenological constants are introduced to characterize the events involved in intracapillary exchange. One of the obvious shortcomings with this approach is that the "appropriate" values for the lumped, effective parameters are difficult to estimate, and the "appropriate" values change when the operating conditions change. In addition, this approach is inappropriate due to the fact that it does not include interactions of the different processes involved. Because these models do not incorporate the individual events which are important, it is difficult to quantitate the impact of alteration of an individual step or the interdependence among the many simultaneous processes.

Treating blood as a continuum with all the transferring species in local chemical equilibrium proves to be reasonably satisfactory for predicting $O_2$ transfer during blood oxygenation accompanied by simultaneous CO$_2$ elimination. However, this approach significantly underpredicts the amount of $O_2$ transport during blood deoxygenation accompanied by CO$_2$ uptake. This poor performance is attributed to the fact that the Bohr effect (alteration of $P_{50}$ by collateral CO$_2$ transport) which has an important effect on the rate of deoxygenation is not taken into account in the continuum model. Furthermore, the continuum approach is determined here to be a poor representation for the CO$_2$ transfer network. This was clearly demonstrated when the predictions of the discrete and continuum models were compared with Voorhees'
experimental data. The error of the continuum model can be attributed to neglecting the fact that blood is a two-compartment system and that each compartment has very different reaction and buffering characteristics. It is observed that certain chemical reaction rates have substantial effects on the pH changes (e.g., extracellular CO₂ hydration/dehydration reactions and HCO₃⁻/Cl⁻ exchange kinetics) but modify the overall gas exchange to a lesser extent. The continuum model is inadequate for prediction of Δ[CO₂]_{total}, and even less adequate for prediction of intra- and extracellular ΔpH values which are important with respect to the acid/base status of blood.

Generally speaking, we find that appropriate conclusions depend on the choice of a proper model and that it is important to take into account the two-phase, compartmentalized nature of blood when describing simultaneous gas exchange and pH regulation by blood flowing in microvessels. The discrete model developed here still needs to be critically validated over a range of diameters. Simultaneous development of theoretical models and experimental measurements is, certainly, an excellent way to further our understanding of the complexities of this transport system.
CHAPTER 7

FUTURE DIRECTIONS

Theoretical Refinement and Experimental Validation of the Mathematical Model

Refinements can be introduced to improve the mathematical model developed in Chapter 6. Firstly, updated and more sophisticated blood-gas equilibria for describing the coupled interactions between hemoglobin, O\textsubscript{2}, CO\textsubscript{2}, H\textsuperscript{+} and DPG should be implemented to the model if available. Secondly, a major undetermined feature of the kinetic equation for HCO\textsubscript{3}\textsuperscript{-}/Cl\textsuperscript{-} across the RBC membrane which is the ratio of in-to-out and out-to-in translocation rate constants should be investigated further. Knauf and Mann (1984) proposed that the intrinsic association constants for anions are the same at both membrane surface and suggested that the translocation rates vary with the direction. They arrived at this conclusion in the view of the fact that the asymmetry of anion-bound sites was equal to that for empty sites. For a ping-pong model, the consideration of the asymmetry of band 3 protein will influence the determinations of the macroscopically dissociation constants of the ions.

Critical testing of the discrete model for predicting O\textsubscript{2}/CO\textsubscript{2} transport and pH regulation over a range of diameters (20 - 100 \textmu m) needs to be carried out. The \textit{in vitro} microvessel microspectrophotometer system developed in this research program (developed by Boland \textit{et al.} (1987) and currently being modified by McKay and Hellums) allows on-line determination of O\textsubscript{2} flux to and from hemoglobin solutions and RBC suspensions under physiological relevant conditions. Consequently, pH indicator (fluorescent probe or microelectrode) and \( P_{CO_2} \) indicator techniques need to be incorporated to the system to allow experimental validation of the model. In addition to a
wide range of diameters, these measurements need to be extended to a range of flow rates, hematocrit and biochemical status of blood samples.

**Application of the Experimental and Theoretical Methodologies to Study Gas Transport and Acid/Base Balance by Biochemically Altered Cells, Pathological Cells and RBC Substitutes; and Effect of Drugs on Blood Respiratory Functions**

The experimental measurements and theory can be extended to analyze gas transport to and from biochemically altered RBCs, pathological RBCs and RBC substitutes; and to study the effect of drugs. By observing the changes in gas transport and acid/base balance and analyzing data obtained with the help of the model, the key parameters which are affected, which in turn modify the gas transport, can be identified and quantified.

**Biochemically Altered RBCs**

Several factors involving RBC morphology and physiology influence the rates of its gas transport and \( \text{pH} \) regulation properties. Cell size and shape define the surface area available for \( \text{O}_2/\text{CO}_2 \) and \( \text{HCO}_3^-/\text{Cl}^- \) exchange across the RBC membrane. Intracellular hemoglobin concentration determines the \( \text{O}_2 \) carriage capacity and intracellular buffering capacity. Changes in intracellular DPG concentration and/or \( \text{pH} \) shift the hemoglobin-\( \text{O}_2 \)-\( \text{CO}_2 \) equilibrium relationship. Alterations in the content of intracellular carbonic anhydrase affect the degree of \( \text{CO}_2 \) retention in blood. The effect of cell size and shape can be studied by measuring the rates of gas exchange of osmotically altered human RBCs or RBCs from other species. The effects of altering intracellular contents of several determining factors can be studied by using reconstituted RBCs tailored made to meet the specified compositions.

**Pathological RBCs**

The model can be applied to examine gas transport by blood in certain pathological cases. For instance, RBCs from patients with sickle cell anemia may be
studied. Findings that polymerization of hemoglobin S is an O₂-linked function, and that
CO₂ has an O₂-affinity independent effect on deoxyhemoglobin S polymerization
suggest that varying P_{CO₂} might have different effects on respiratory functions and other
RBC properties of blood in sickle cell anemia compared with normal blood. Part of the
low affinity associated with sickle cell anemia can be explained by elevated levels of
DPG, as seen with many anemic states. In addition, it was reported that the CO₂-
induced Bohr effect of sickle cell blood is considerably larger than normal (Ueda and
Bookchin, 1984) which also contributes to the substantially lowering of the O₂ affinity.
As another example, blood from patients with the conditions of either respiratory
acidosis or alkalosis may be studied. Normal physiological processes occur only within
a narrow range of pH; however, in a number of diseases the acid/base balance may be
shifted severely away from the normal acid/base environment. Respiratory acidosis
(alkalosis) is an abnormal condition caused by the presence of excessive amounts of acid
(alkali) or by reduction of alkali (acid) to a subnormal level; and the acid concerned in the
disturbance is H₂CO₃.

RBC Substitutes

Another application of these experimental and mathematical methodologies is to
evaluate the efficiency of various RBC substitutes in delivering O₂ and removing CO₂.
Three classes of synthetic blood substitutes that are presently being tested in various
laboratories include perfluorochemical emulsion, crosslinked or polymerized hemoglobin
molecules free in solution and artificial RBCs prepared by encapsulating hemoglobin
inside lipid bilayer vesicles. These blood substitutes have been studied primarily from
an empirical point of view in terms of life support, circulation half-life, shelf life and
safety with regard to pyrogens in whole animal transfusion studies. Therefore, the
contributions which our experimental and theoretical methods can make is to provide
systematic and quantitative analyses on the gas transport properties of these blood
substitutes. For example, transport property of liposome-encapsulated hemoglobin can be measured and analyzed as a function of vesicle size, lipid composition and intracellular compositions (Olson, personal communication).

*Effect of Drugs*

The key parameters which are modified by drugs, which in turn affect the gas transport and acid/base change of blood can be studied by simulation of the experiments which use the drugs. For example, one can study modification of the $O_2$ affinity of hemoglobin and normalization of the Bohr effect by some antisickling agents proposed for possible therapeutic roles in sickle cell anemia, such as potassium cyanate. One can also investigate the physiological alterations on CO$_2$ transport and deviations from the normal state of acid/base balance due to the presence of pharmacological agents, such as furosemide which inhibit RBC anion exchange and carbonic anhydrase activity, or acetazolamide, a more potent inhibitor of carbonic anhydrase activity.

*Application of the Experimental and Theoretical Methodologies to Improve the Design of Extracorporeal Blood Oxygenator*

Membrane oxygenators used in cardiopulmonary bypass surgery and in membrane lungs involve oxygen transport from relatively large conduits (about 100 $\mu$m and larger), and in most of these devices the mass transfer resistance attributable to the blood phase is a significant fraction of the total resistance. Hence, these experimental and mathematical approaches have utility in designing oxygenators and aiding in the interpretation of membrane lung function. In addition, more experimental work is needed to better quantify the $O_2$ transfer augmentation in blood and the validity of the model presented in Chapter 4 for shear-induced augmentation of $O_2$. Furthermore, with additional experimental results a more correct model of augmentation would be appropriate. Finally, a study can be conducted to evaluate the feasibility of oxygenator designs which take advantage of the effects of shear-induced augmentation of $O_2$. 
REFERENCES


New York: Plenum Press.


APPENDIX A

Validation of Space Average Oxygen Saturation Calculation Involved in Simulation of Boland et al.'s System

In Boland et al.'s dual wavelength microspectrophotometric system, the extent of oxygenation of the RBC suspension was monitored by measuring absorbance changes at two different wavelengths. Because of the small path length (30 μm) of the absorbing solution, measurements were made at the Soret region wavelengths of 414 and 430 nm; these wavelengths represent the absorption maxima of oxy- and deoxyhemoglobin, respectively. The dual wavelength measurements were carried out on fully saturated (100%) and unsaturated (0%) specimens flowing under the same conditions as the test specimen (exp). All light intensity measurements were made after the steady-state condition was reached. The change in absorbance at each wavelength, ΔA_{exp-100%}^{430} or ΔA_{exp-100%}^{414}, was calculated from the negative logarithm of the ratio of the transmittance voltage of the sample under the O₂ transport condition to that for the fully oxygenated sample. The ΔA_{exp-100%} values at each wavelength were then subtracted to reduce light scattering artifacts and to enhance the resultant signals, since the absorbance changes are in opposite directions. The change in absorbance in passing from the fully deoxygenated state to the fully oxygenated state at both wavelengths are ΔA_{0%-100%}^{430} and ΔA_{0%-100%}^{414}.

The difference between ΔA_{0%-100%} at 430 and 414 nm represents the maximum possible signal; this quantity was used to normalize the difference between ΔA_{exp-100%}^{430} and ΔA_{exp-100%}^{414} and to compute the fractional degree of saturation of hemoglobin in the following equation:

\[
(\bar{S})_{measured} = 1 - \frac{\Delta A_{exp-100%}^{430} - \Delta A_{exp-100%}^{414}}{\Delta A_{0%-100%}^{430} - \Delta A_{0%-100%}^{414}}
\]  
(A.1)
which is equivalent to Equation (A.2a) or (A.2b).

\[
\langle S \rangle_{\text{measured}} = 1 - \frac{\ln \left( \frac{V_{100\%}^{430}}{V_{100\%}^{414}} \right)}{\ln \left( \frac{V_{100\%}^{430}}{V_{0\%}^{430}} \right)} - \ln \left( \frac{V_{100\%}^{414}}{V_{0\%}^{414}} \right)
\]

(A.2a)

\[
\langle S \rangle_{\text{measured}} = 1 - \frac{\ln \left( \frac{q_{100\%}^{430}}{q_{100\%}^{430}} \right)}{\ln \left( \frac{q_{100\%}^{430}}{q_{0\%}^{430}} \right)} - \ln \left( \frac{q_{100\%}^{414}}{q_{0\%}^{414}} \right)
\]

(A.2b)

where \( V \)'s and \( q \)'s are the transmittance voltage and light intensity at different wavelengths and conditions, respectively.

In comparison of theoretical results with experimental results, the radially varying \( O_2 \) saturations from the mathematical model must be averaged over the cross section in a suitable fashion. In analyzing Boland et al.'s data, space averaged \( O_2 \) saturations from the theory are calculated so that the comparison can be made directly with the microspectrophotometric determinations of \( O_2 \) saturations. The space average saturation is calculated as

\[
\langle S \rangle_{\text{sa}} = \frac{1}{\pi r_T^2 H_T} \int_0^{r_c} 2 \pi r h(r) S(r) \, dr
\]

(A.3)

The primary objective of the following calculation is to rigorously validate the above averaging method. It is proposed to use the \( O_2 \) saturation profile, \( S(r) \), generated from the model and the basic equation for describing energy transport in absorbing media to
calculate the experimentally measured absorbance. As a result, it is then possible to directly compare the average O₂ saturation calculated by Equations (A.2b) and (A.3).

In order to describe energy transport in nontransparent media, Bird et al. (1960) wrote differential equations for the local rate of energy as viewed both from the material and the radiation standpoint. For the electromagnetic radiation phase, they wrote the following equation for describing the local rate of radiant-energy density U:

\[
\frac{\partial U}{\partial t} = - (\nabla \cdot q) + (E - \psi)
\]  

(A.4)

where

\( q \) is the radiant energy flux.

\( E \) is the energy lost by the material phase resulting from the emission of photons by molecules of the material phase.

\( \psi \) is the local gain of energy by the material phase resulting from photon absorption by the molecules of the material phase.

Equation (A.4) can also be written for the radiant energy within a frequency range \( \lambda \) to \( \lambda + d\lambda \):

\[
\frac{\partial U^\lambda}{\partial t} = - (\nabla \cdot q^\lambda) + (E^\lambda - \psi^\lambda)
\]  

(A.5)

The conditions which are applicable to Boland et al.'s spectrophotometric system are described below. It is a steady-state system in which a monochromatic radiant beam of frequency \( \lambda \), focused parallel to the y-axis, passes through an absorbing fluid (see Figure A.1). In addition, the absorbing media can be assumed to be at temperatures sufficiently low enough that emission by the media is unimportant. Furthermore, the local rate of volumetric energy absorption, \( \psi^\lambda \), is given by \( m^\lambda q^\lambda \), in which \( m^\lambda \) is the
extinction coefficient of the pigment in the medium at frequency \( \lambda \). Then Equation (A.5) becomes

\[
\frac{dq^\lambda}{dy} = -\psi^\lambda
\]  

(A.6)

Rearranging Equation (A.6) and integration with respect to \( y \) gives

\[
\ln \left( \frac{q^\lambda}{q^\lambda_{ref}} \right) = \int_{y = -\sqrt{r^2 - x^2}}^{y = \sqrt{r^2 - x^2}} m^\lambda \, dy
\]

(A.7)

where

\( q^\lambda \) is the light intensity at wavelength \( \lambda \) after passing through the medium.

\( q^\lambda_{ref} \) is the incident light at wavelength \( \lambda \).

\( x \) and \( y \) are the coordinate system (see Figure A.1).

![Diagram of cross section of the vessel](image)

cross section of the vessel

direction of optic axis

**Figure A.1:** Explanation of coordinates.
If the system contains two non-interacting pigments, oxygenated and deoxygenated hemoglobin, the concentrations of oxygenated hemoglobin ([HbO₂]) and deoxyhemoglobin ([Hb]) can be related to their respective extinction coefficients at wavelength λ (ε_HbO₂^λ and ε_Hb^λ),

\[ m^λ = ε_{HbO₂}^λ [HbO₂] + ε_{Hb}^λ [Hb] \]  \hspace{1cm} (A.8)

Equation (A.8) can be rewritten in terms of O₂ saturation and hemoglobin concentration.

\[ m^λ(r) = \left[ ε_{HbO₂}^λ S(r) + ε_{Hb}^λ [1 - S(r)] \right] C_{Hb, rbc} h(r) \]  \hspace{1cm} (A.9)

where \( C_{Hb, rbc} \) is the intracellular hemoglobin concentration, and \( h(r) \) is the hematocrit profile. Because the experimental determinations were obtained over a rectangular field of view of approximately 28x40 μm to yield factional saturation of hemoglobin, one needs to integrate Equation (A.7) with respect to x over the domain of \( x=-r_c \) to \( x=r_c \).

\[ \left\langle \ln \left( \frac{q^λ}{q^λ_{ref}} \right) \right\rangle = \frac{C_{Hb, rbc}}{2 r_c} \int_{x=-r_c}^{x=r_c} \int_{y=\sqrt{r_c^2-x^2}}^{y=\sqrt{r_c^2-x^2}} \left[ ε_{HbO₂}^λ S(r) + ε_{Hb}^λ [1 - S(r)] \right] h(r) \, dy \, dx \]  \hspace{1cm} (A.10)

The above integral can be more easily evaluated by changing to polar coordinate.

\[ \left\langle \ln \left( \frac{q^λ}{q^λ_{ref}} \right) \right\rangle = \frac{C_{Hb, rbc}}{r_c} \int_{θ=-\frac{π}{2}}^{θ=\frac{π}{2}} \int_{r=0}^{r=r_c} \left[ ε_{HbO₂}^λ S(r) + ε_{Hb}^λ [1 - S(r)] \right] h(r) \, r \, dr \, dθ \]  \hspace{1cm} (A.11)

Therefore, the quantities listed in Equation (A.12) can then be calculated using either Equation (A.10) or (A.11); \( \langle S \rangle_{measured} \) can then be computed using Equation (A.2b).
\[ \left( \frac{q_{\text{exp}}}{q_{\text{ref}}} \right), \left( \frac{q_{100\%}}{q_{\text{ref}}} \right), \text{and} \left( \frac{q_{0\%}}{q_{\text{ref}}} \right) \quad \text{for } \lambda = 430 \text{ and } 415 \text{ nm}. \]  \hspace{1cm} (A.12)

Given \( h(r) \) and \( S(r) \) for situations of either \( O_2 \) uptake or release experiments (runs R1, R2, U1 and U2 presented in Figures 4.1 - 4.4), the comparison of the \( O_2 \) saturation computed via Equation (A.2b) is plotted against the \( O_2 \) saturation computed via Equation (A.3). The fact that all the points fall on the 45\(^\circ\) line indicates Equation (A.3) is an appropriate method for calculating space-average \( O_2 \) saturation.
Figure A.2: Comparison of $O_2$ saturation values computed by two independent methods. The ordinate is $O_2$ saturation calculated via Equation (A.3), and the abscissa is $O_2$ saturation calculated via Equation (A.2b). Results are given for Boland et al.'s experimental data which are presented in Figures 4.1 - 4.4.
APPENDIX B

Estimation of the Extracellular Mass Transfer Coefficient of CO₂
in A Stopped-flow Rapid-mixing Apparatus

Evidence which supports the fact that unstimred solvent layers account for almost
entirely for the small mass transfer coefficient for O₂ at the cell surface in a stopped-flow
apparatus had been discussed by Coin and Olson (1979). As pointed out by Vandegriff
and Olson (1984a) that the magnitude of the external diffusion effect can be estimated
from a simple boundary analysis:

\[ k_{O₂} = \frac{α_{p,O₂} D_{O₂,pl}}{Δx} \]  \hspace{1cm} (B.1)

where

\( k_{O₂} \) and \( D_{O₂,pl} \) are the extracellular mass transfer coefficient and extracellular
diffusivity of \( O₂ \), respectively.

\( α_{p,O₂} \) is the partition coefficient of \( O₂ \) between the phases, and \( α_{p,O₂}=1 \).

\( Δx \) represents the thickness of the unstimred layers.

Furthermore, Vandegriff and Olson (1984a) examined \( O₂ \) uptake by RBCs in a stopped-
flow rapid-mixing experiments by developing a detailed three-dimensional model and
using previously developed hydrodynamic theories to describe the unstimred solvent
layer. An initial 1 μm unstimred layer was postulated to occur during mixing and
expanded with time by a \((t)^{0.5}\) function when flow stops. This formula in combination
with their cylindrical disk model was reported to fit a wide range of experimental
conditions. Their result indicated that there is much less turbulent mixing after flow
stops and that the diffusion sublayer is between 8-20 μm during most of the reaction
they studied. If the properties in Equation (B.1) were replaced by that of CO₂, $D_{CO₂,pl} = 1.85 \times 10^{-5}$ cm²/sec and $\Delta x$ taken to be $= 14$ μm, $k_{CO₂}$ is then calculated to be $\approx 0.013$ cm/sec.

In this study, to simplify the mathematical analysis and neglect the detailed description of the incomplete stirring layer adjacent to the cell surface, the flux of CO₂ across the boundary layer which includes the RBC membrane and the unstirred layer is written as the followings:

$$Flux_{CO₂} = \eta_{CO₂} \left[ \frac{[CO₂]_{rbc} - [CO₂]_{pl}}{\alpha_{CO₂,rbc} - \alpha_{CO₂,pl}} \right]$$

(B.2)

where

$\eta_{CO₂}$ is the lumped mass transfer coefficient for CO₂, and it is related to $k_{CO₂}$ through the following relationship: $\eta_{CO₂} = k_{CO₂} \alpha_{CO₂,pl}$. $\alpha_{CO₂,pl}$ is the CO₂ solubility coefficient in the extracellular phase, and in this equation it has the unit of (mmHg)$^{-1}$.

$\alpha_{CO₂,rbc}$ and $\alpha_{CO₂,pl}$ are the intracellular and extracellular solubility coefficients for CO₂, respectively. $\alpha_{CO₂,pl}$ is related to $\alpha_{CO₂,pl}$ through the molar volume of CO₂.

From Equation (B.1), a simple boundary analysis, $\eta_{CO₂}$ is estimated to be $\approx 8 \times 10^{-6}$ cm/sec/mmHg. Additionally, the magnitude of this external diffusion effect is also calculated from the following two methods. The first approach incorporates Kagawa and Mochizuki's calculation for $\eta_{O₂}$ which was obtained from modeling the oxygenation rate of RBCs in a stopped-flow reactor (Kagawa and Mochizuki, 1982). The second method involves the usage of the low Reynolds number asymptotic solution for diffusion from a disc to the surrounding fluid.
Kagawa and Mochizuki's calculation

This differential equations which consists of two components, e.g., the diffusion and chemical reaction components, as given in a two-dimensional cylindrical-disk model by Equation (B.3) was used by Kagawa and Mochizuki (1982) to estimate $\eta_{O2}$.

$$\left(1 + \frac{d[HbO_2]_{rbc}}{d[O_2]_{rbc}}\right) \frac{\partial [O_2]_{rbc}}{\partial t} = D_{O2,rbc} \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial [O_2]_{rbc}}{\partial r} \right) + \frac{\partial^2 [O_2]_{rbc}}{\partial x^2} \right]$$

(B.3)

where

$[O_2]_{rbc}$ and $[HbO_2]_{rbc}$ are the dissolved $O_2$ and oxyhemoglobin concentrations in the RBC, respectively.

d$[HbO_2]_{rbc}$/$d[O_2]_{rbc}$ is the slope of the ODC.

$D_{O2,rbc}$ is the diffusivity for $O_2$ in the RBC.

They used the $O_2$ transfer data obtained by Mochizuki's (1966) which was obtained from stopped-flow rapid-reaction apparatus to estimate $\eta_{O2}$. Because of the symmetricity of radial and vertical diffusion, the $P_{O2}$ gradient at $r=0$ and $z=0$ becomes zero; and the diffusion rate across the boundary layer was expressed by the transfer coefficient, $\eta_{O2}$. Let the distances of the origin be $r=c$, and $z=\pm d$, respectively. The boundary conditions were given by

$$D_{O2,rbc} \frac{\partial [O_2]_{rbc}}{\partial r} \bigg|_{r=R_{rbc}} = \eta_{O2} (P_{O2} \bigg|_{r=R_{rbc}} - P_{O2,pl})$$  \hspace{1cm} (B.4a)

$$D_{O2,rbc} \frac{\partial [O_2]_{rbc}}{\partial r} \bigg|_{z=\pm t_{rbc}} = \eta_{O2} (P_{O2} \bigg|_{z=\pm t_{rbc}} - P_{O2,pl})$$  \hspace{1cm} (B.4b)
where

\[ R_{rbc} \] is the RBC radius (3.5 \, \mu m).

\[ t_{rbc} \] is the RBC thickness (1.6 \, \mu m).

\[ P_{O2,pl} \] is the \( P_{O2} \) in the surrounding medium; \( P_{O2,pl} \) can be taken as approximately constant because volume ratio of suspending buffer to RBCs was \( \gg 1 \) Mochizuki’s experiment.

Kagawa and Mochizuki (1982) computed the oxygenation process by varying the \( \eta_{O2} \) value, while initial and boundary conditions were given according to Mochizuki’s data (1966). According to them, the \( O_2 \) saturation-time curve calculated by use of \( \eta_{O2} = 2.5 \times 10^{-6} \, \text{cm/sec/mmHg} \) coincided the best with the experimental data.

The above approach is obviously an oversimplification of the real situation in stopped-flow rapid-mixing experiments. Even after convective mixing had ceased to occur, the \( O_2 \) concentration gradient in the external aqueous layer is not a linear function of the distance from the cell surface (Vandegriff and Olson, 1984a). In addition, the thickness of the unstirred layer varied with the time. In the initial turbulent flow within the mixing chamber, the cells were well mixed and only a thin layer of solution surrounding the cell surface remained unstirred. As flow continued into the observation chamber and the turbulence began to dissipate, the cells became entrained in microscopic eddies that grew even larger after laminar flow stopped. The unstirred layer is empirically determined to be projecting in the following manner (Vandegriff and Olson, 1984c):

\[ d(t) = d(0) + K t^{0.5} \]  \hspace{1cm} (B.5)

where

\[ d(t) \] is the thickness of the unstirred layer at time \( t \).
\( d(0) \) is the thickness of the unstimred layer during turbulent flow in the mixer.

\( K \) is a proportionality constant and has to be determined empirically; and

\[ K = R_{rbc}^{1/3} D_{O2}^{1/3} \nu^{7/12} \]

where \( \nu \) is the kinematic viscosity.

However, for a first order approximation, the detailed description of incomplete stirring of the aqueous layer adjacent to cell surface is neglected. The mass transfer resistance to O\(_2\) that exists at this unstimred layer is assumed to be lumped into the empirical, effective mass transfer coefficient, \( \eta_{O2} \). Due to the lack of stopped-flow data on CO\(_2\) transport, the value of \( \eta_{CO2} \) is taken to be the same as that of \( \eta_{O2} \).

**Low Reynolds number asymptotic solution for diffusion from a disc to the surrounding fluid**

\( \eta_{CO2} \) can also be approximated from Nusselt number correlations for mass transfer from disc to the surrounding fluid (Merchuk et al., 1983)

\[ \frac{k_{CO2} t_{rbc}}{D_{CO2,pl}} = \frac{8}{\pi} \]

(B.6)

where

\( k_{CO2} \) is the extracellular mass transfer coefficient of CO\(_2\), and it is related to \( \eta_{CO2} \) through the following relationship:

\[ \eta_{CO2} = k_{CO2} \alpha_{CO2,pl} \]

\( t_{rbc} \) is the thickness of the RBC.

The \( 8/\pi \) term is the asymptotic value of the Nusselt number for pure diffusion from the disc to a stationary medium around it. From Equation (B.6), \( \eta_{CO2} \) is calculated to be about \( 6 \times 10^{-6} \) cm/sec/mmHg.
APPENDIX C

Dedimensionalization of Type I Discrete Model Which Consists of

Equations (6.1.1) - (6.1.10)

In this section, Type I discrete model, Equations (6.1.1) - (6.1.10), for describing simultaneous O₂/CO₂ transport and acid/base regulation by blood flowing in microvessels is dedimensionalized. It is convenient to introduce the following dimensionless variables [given in Equation (6.33)]:

\[ t = \frac{z}{L}, \quad x = \frac{r}{r}, \quad x' = \frac{r_c - r}{r_c}, \quad x'' = \frac{r_c}{r} \]

\[ u = \frac{[O_2]_{rbc}}{[O_2]_o}, \quad v = \frac{[CO_2]_{rbc}}{[CO_2]_o}, \quad w = \frac{[H^+]_{rbc}}{[H^+]_o}, \quad s = \frac{[Cl^-]_{rbc}}{[Cl^-]_o}, \]

\[ w' = \frac{[H^+]_{pl}}{[H^+]_o}, \quad r' = \frac{[HCO_3^-]_{pl}}{[HCO_3^-]_o}, \quad s' = \frac{[Cl^-]_{pl}}{[Cl^-]_o}, \]

\[ v'' = \frac{[CO_2]_{pl}}{[CO_2]_o}, \quad w'' = \frac{[H^+]_{pl}}{[H^+]_o}, \quad r'' = \frac{[HCO_3^-]_{pl}}{[HCO_3^-]_o} \]

When the equations of continuity are written in terms of these dimensionless variables, we obtain Equations (C.1) - (C.10).
\[
\left(\frac{\alpha_{O_2, rbc}}{\alpha_{O_2, pl}}\right) h(x) D (1 - \text{slp}) (1 - Bb^2 x^2) \left[ 1 + \frac{C_{\text{heme, rbc}}}{[O_2]_o} \frac{\partial S}{\partial u} \right] + (1 - h(x)) D (1 - Bb^2 x^2) \left( \frac{[O_2]_o}{L} \right) \frac{\partial u}{\partial t} = D_{O_2, pl} \alpha_{O_2, pl} \frac{1}{r^2} \frac{\partial}{\partial x} \left( x \frac{\partial u}{\partial x} \right)
\]
(C.1)

\[
\left(\frac{D_{CO_2, pl}}{r^2}\right) \left[ \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \right] h(x) D (1 - \text{slp}) (1 - Bb^2 x^2) \left[ 1 + \frac{C_{\text{heme, rbc}}}{4[CO_2]_o} \frac{\partial \phi}{\partial v} + \left( \frac{K'}{f_{\text{water}}} \right) \frac{1}{w} \right] + \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \left(1 - h(x)\right) D (1 - Bb^2 x^2) \left( \frac{[CO_2]_o}{L} \right) \frac{\partial v}{\partial t} = \left(\frac{D_{CO_2, pl}}{r^2}\right) \left[ \frac{\alpha_{CO_2, pl}}{\alpha_{CO_2, rbc}} \right] \frac{1}{x} \frac{\partial}{\partial x} \left( x \frac{\partial v}{\partial x} \right) + h(x) \left(\frac{\xi}{v}\right)_{rbc} \text{Flux}_{HCO_3} - (1 - h(x)) R_{HCO_3, pl}
\]
(C.2)

\[
D (1 - \text{slp}) (1 - Bb^2 x^2) \left[ \left( \frac{\beta_{rbc}}{2.303 [H^+]_o} \right) \frac{1}{w} + \left( \frac{K'}{f_{\text{water}}} \right) \frac{[CO_2]_o}{[H^+]_o} \frac{v}{w^2} - 2 \right] \left( \frac{[H^+]_o}{L} \right) \frac{\partial w}{\partial t} = - \left(\frac{\xi}{v}\right)_{rbc} \text{Flux}_{HCO_3}
\]
(C.3)

\[
D (1 - \text{slp}) (1 - Bb^2 x^2) \left( \frac{[Cl^-]_o}{L} \right) \frac{\partial s}{\partial t} = - \left(\frac{\xi}{v}\right)_{rbc} \text{Flux}_{HCO_3}
\]
(C.4)

\[
(1 - h(x)) D (1 - Bb^2 x^2) \left( \frac{[H^+]_o}{L} \right) \frac{\partial w'}{\partial t} = D_{H^+, pl} \left( \frac{[H^+]_o}{L} \right) \frac{1}{r^2} \frac{\partial}{\partial x} \left( x \frac{\partial w'}{\partial x} \right) + \left( \frac{2.303 [H^+]_o}{\beta_{pl}} \right) (1 - h(x)) \left( \frac{[H^+]_o}{L} \right) \left( \frac{\partial w'}{\partial t} \right) R_{HCO_3, pl}
\]
(C.5)

\[
(1 - h(x)) D (1 - Bb^2 x^2) \left( \frac{[HCO_3^-]_o}{L} \right) \frac{\partial r'}{\partial t} = D_{HCO_3, pl} \left( \frac{[HCO_3^-]_o}{L} \right) \frac{1}{r^2} \frac{\partial}{\partial x} \left( x \frac{\partial r'}{\partial x} \right) - h(x) \left(\frac{\xi}{v}\right)_{rbc} \text{Flux}_{HCO_3} + (1 - h(x)) R_{HCO_3, pl}
\]
(C.6)

\[
(1 - h(x)) D (1 - Bb^2 x^2) \left( \frac{[Cl^-]_o}{L} \right) \frac{\partial s'}{\partial t} = D_{Cl, pl} \left( \frac{[Cl^-]_o}{L} \right) \frac{1}{r^2} \frac{\partial}{\partial x} \left( x \frac{\partial s'}{\partial x} \right) + h(x) \left(\frac{\xi}{v}\right)_{rbc} \text{Flux}_{HCO_3}
\]
(C.7)
\[ A(1-b)[2-(1-b)x']x' \left( \frac{[CO_2]_o}{L} \right) \frac{\partial v''}{\partial t} = \left[ \frac{D_{CO_2,pl}[CO_2]_o}{(r_c-r_r)^2} \right] \frac{\partial^2 v''}{\partial x^2} - \left[ \frac{D_{CO_2,pl}[CO_2]_o}{(r_c-r_r)(r_c-r_r')x'}(r_c-r_r') \right] \frac{\partial v''}{\partial x'} - R'_{HCO_3,pl} \]

\[ A(1-b)[2-(1-b)x']x' \left( \frac{[H^+]_o}{L} \right) \frac{\partial w''}{\partial t} = \left[ \frac{D_{H^+,pl}[H^+]_o}{(r_c-r_r)^2} \right] \frac{\partial^2 w''}{\partial x^2} - \left[ \frac{D_{H^+,pl}[H^+]_o}{(r_c-r_r)(r_c-r_r')x'}(r_c-r_r') \right] \frac{\partial w''}{\partial x'} + \left( \frac{2.303 [H^+]_o}{\beta_{pl}} \right) w'' R'_{HCO_3,pl} \]

\[ A(1-b)[2-(1-b)x']x' \left( \frac{[HCO_3^-]_o}{L} \right) \frac{\partial r''}{\partial t} = \left[ \frac{D_{HCO_3,pl}[HCO_3^-]_o}{(r_c-r_r)^2} \right] \frac{\partial^2 r''}{\partial x^2} - \left[ \frac{D_{HCO_3,pl}[HCO_3^-]_o}{(r_c-r_r)(r_c-r_r')x'}(r_c-r_r') \right] \frac{\partial r''}{\partial x'} + R'_{HCO_3,pl} \]

where

\[ h(x) = h_m(1-x^m) \]

If modified Hill model is used, then

\[ \frac{\partial S}{\partial u} = n \left\{ \frac{[O_2]_o}{C_{50}} \right\}^n u^{n-1} \left[ 1 + \left( \frac{[O_2]_o}{C_{50}} \right)^n u^n \right] \]

If modified Easton model is used, then

\[ \frac{\partial S}{\partial u} = \left\{ \frac{\kappa [O_2]_o}{\alpha_{O_2,rbc}} \right\} (S_{max} - S_{min}) \exp \left[ \kappa P_{O_2} - \left( \frac{\kappa [O_2]_o}{\alpha_{O_2,rbc}} \right) u \right] \exp \left[ \kappa P_{O_2} - \left( \frac{\kappa [O_2]_o}{\alpha_{O_2,rbc}} \right) u \right] \]

\[ \frac{\partial \phi}{\partial v} = \frac{2 \lambda_\alpha [CO_2]_o + 4 \lambda_\alpha^2 [CO_2]_o^2 v}{\left( 1 + \lambda_\alpha [CO_2]_o v \right)^2} + \frac{2 \lambda_\beta [CO_2]_o (1 + K_{DPG}[DPG]) + 4 \lambda_\beta^2 [CO_2]_o^2 v}{\left( 1 + \lambda_\beta [CO_2]_o v + K_{DPG}[DPG] \right)^2} \]
\[
\text{Flux}_{\text{HCO}_3} = \frac{T_{\text{tot}} k_{\text{trans}} K_A [\text{HCO}_3^-]_o (s' r' - s' r)}{(s + s') + \left(\frac{[\text{HCO}_3^-]_o}{[\text{Cl}^-]_o}\right)(r + r') + 2 \left(K_A [\text{Cl}^-]_o s s' + \left(\frac{K_A [\text{HCO}_3^-]_o^2}{[\text{Cl}^-]_o}\right)r' r + (K_A [\text{HCO}_3^-]_o)(s' r' + s' r)\right)}.
\]

\[
R_{\text{HCO}_3,p} = \tilde{\lambda}_{pl} \left[ k_u \left( \frac{\alpha_{\text{CO}_2,p}}{\alpha_{\text{CO}_2,rbc}} \right) [\text{CO}_2]_o v - \left( \frac{k_v}{K_1} \right) [H^+]_o [\text{HCO}_3^-]_o w' r' \right]
\]

\[
R_{\text{HCO}_3,p} = \tilde{\lambda}_{pl} \left[ k_u [\text{CO}_2]_o v'' - \left( \frac{k_v}{K_1} \right) [H^+]_o [\text{HCO}_3^-]_o w'' r'' \right]
\]

Rewriting the above equations in terms of the dimensionless groups (\(a's, d's, u_{\text{ext}}\) and \(v_{\text{ext}}\) which are listed in Section 6.1.4) yields Equations (6.34) - (6.43).

For the discrete model, mixed mean concentrations of species i inside the RBC ([C_i]_{mn,rbc}), in the plasma ([C_i]_{mn,pl}) and in the whole blood ([C_i]_{mn,wb}) are calculated by Equations (6.73a), (6.73b) and (6.73c), respectively. By introducing the normalization quantities listed in Equation (C.11), Equations (6.73a) - (6.73c) can be rewritten in terms of the dimensionless variables.

\[
C_i = \frac{[C_i]_{rbc}}{[C_i]_o}, \quad C_i' = \frac{[C_i]_{pl}}{[C_i]_o}, \quad C_i'' = \frac{[C_i]_{wb}}{[C_i]_o}, \quad x = \frac{r_c}{r}, \quad x' = \frac{r_c - r}{r_c - r_c'} \quad \text{and} \quad b = \frac{r}{r_c}
\]  

(C.11)
Normalized mixed mean concentration of species i inside the RBC

\[
\frac{\langle C_i \rangle_{mm, rbc}}{[C_i]_o} = \frac{2b^2 h_m D (1-slp)}{HT \langle V \rangle_{rbc}} \int_0^1 x (1 - x^m)(1 - Bb^2 x^2) C_i(x) \, dx
\]  
(C.12)

Normalized mixed mean concentration of species i in the plasma

\[
\frac{\langle C_i \rangle_{mm, pl}}{[C_i]_o} = \frac{2b^2 D}{(1 - H_T) \langle V \rangle_{pl}} \int_0^1 x [1 - h_m(1 - x^m)](1 - Bb^2 x^2) C_i'(x) \, dx
\]
\[+ \frac{2(1-b)^2 A}{(1 - H_T) \langle V \rangle_{pl}} \int_0^1 x' [1 - (1 - b)x'][2 - (1 - b)x'] C_i''(x') \, dx'
\]  
(C.13)

Normalized mixed mean concentration of species i inside the vessel

\[
\frac{\langle C_i \rangle_{mm, wb}}{[C_i]_o} = \frac{2b^2 h_m D (1-slp)}{\langle V \rangle} \int_0^1 x (1 - x^m)(1 - Bb^2 x^2) C_i(x) \, dx + \frac{2b^2 D}{\langle V \rangle} \int_0^1 x [1 - h_m(1 - x^m)](1 - Bb^2 x^2) C_i'(x) \, dx
\]
\[+ \frac{2(1-b)^2 A}{\langle V \rangle} \int_0^1 x' [1 - (1 - b)x'][2 - (1 - b)x'] C_i''(x') \, dx'
\]  
(C.14)