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A quasi-elastic neutron scattering study of hydrogen dynamics in aqueous polymer solutions

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Rice University, 1986
RICE UNIVERSITY

A QUASI-ELASTIC NEUTRON SCATTERING STUDY OF HYDROGEN DYNAMICS IN AQUEOUS POLYMER SOLUTIONS

by

DANIEL W. BEARDEN

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

A QUASI-ELASTIC NEUTRON SCATTERING STUDY OF HYDROGEN DYNAMICS IN AQUEOUS POLYMER SOLUTIONS

by

Daniel W. Bearden

We have used quasi-elastic neutron scattering techniques to study the dynamics of solvent water protons in H$_2$O solutions of deuterated poly(ethylene oxide) (Polyox) at room temperature. Using a slightly modified technique, we also have obtained values for the mean-square vibrational amplitude of polymer protons in powder and D$_2$O solutions of Polyox and trypsin at temperatures between 300K and 75K.

To study the dynamics of solvent water in deuterated Polyox (dPolyox) solutions, we used a high energy-resolution spectrometer ($\Gamma_{res} = 100\mu$eV) so that we could measure the width of the quasi-elastic line for various values of the momentum transfer, Q. The Q-dependence of the quasi-elastic linewidth shows that the diffusive properties of water in these Polyox/water solutions is slightly affected at low (5 polymer wt%) and intermediate (10-20 wt%) polymer concentrations. At high polymer concentration (38 wt%), the water clearly has a reduced diffusion coefficient and an increased residence time compared to bulk water and this may be attributed to the nature of the interaction of the water molecules with the polymer.
We were motivated to study the mean-square vibrational amplitude of protons in powder and D₂O solutions of Polyox and trypsin because of recent computer simulations on biological macromolecules. We measured the vibrational mean-square amplitude by using a low energy-resolution spectrometer (Γ_{res} = 700μeV) to measure the intensity of the quasi-elastic peak in a relatively short time compared to standard high energy-resolution techniques. Our results on these 20 wt% solutions at 300K are consistent with conventional quasi-elastic neutron scattering results on the Polyox and with computer simulations of trypsin in solution. The vibrational amplitude was proportional to temperature throughout the temperature range studied, and the proportionality constant is interpreted in terms of an effective spring constant for the motion of the hydrogens. These spring constants are smaller than those for some common chemical bonds indicating that there are significant contributions to the motion of the hydrogens besides vibrational motion.
ACKNOWLEDGEMENTS

I would like to thank my friends and mentors who helped me finish this thesis.

The skillful insight of Dr. H. E. Rorschach, which has been fashioned through years in the pursuit of an understanding of our physical world, is largely responsible for the effort represented in this thesis. I am truly indebted to him for his patience and guidance through these years. The infectious enthusiasm of Dr. C. F. Hazlewood for the scientific method has given me true understanding of the word "science". I am indebted to both of these professors for their allowing me to work with them.

I would like to thank Dr. G. T. Trammel and Dr. R. L. Sass for their interest in my work and for the time they spent on my thesis committee. I also appreciate the data and support supplied by Dr. J. A. McCammon and Dr. C. F. Wong of the University of Houston.

The people at Oak Ridge National Laboratory were very supportive and helpful. I would like to thank Dr. R. Nicklow and Dr. M. Hagan for their effort in making these experiments possible.

The time I spent working toward this degree would have not been as memorable were it not for my fellow graduate students. I would particularly like to thank Tom Egan, Tim Hutton, Doug Heidorn, and Chen Lin for the many hours of discussion, argument, and companionship they have shared with me. I truly wish them well.

Finally, I would like to express my gratitude to all the members of my family. The support a family gives is like no other, and without that support, I would have fallen short. It is with true affection and respect that I dedicate this thesis to my loving wife, Amy.
This work was supported by the R. A. Welch Foundation, the Texaco Corporation, and the Department of Energy through Oak Ridge Associated Universities.
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INTRODUCTION

A. The relevance of the study of macromolecular systems.

Living things must be able to grow, reproduce and take nourishment from the environment. These activities, which distinguish living things from dead things, are the result of ions, molecules, and macromolecules in solvents, working together to provide the structure and function characteristic of living organisms. Each scientific step toward an understanding of the living state seems to emphasize that without the specialized function of macromolecules in the form of proteins, enzymes (catalytic proteins), or ribonucleic acids for example, life would not be possible.

Although macromolecules are critical elements in living systems, our understanding of the microscopic nature of these large molecules is far from complete. This is not too surprising because even in the case of simple poly-atomic liquids, no general microscopic theory has been formulated. The difficulty with developing a microscopic theory for liquids is due to the non-negligible interatomic potential, the lack of periodic structure, and the many-body nature of the problem. When multi-component liquids or solutions are considered, the problems are compounded. The situation may seem even more hopeless when we introduce molecules which may be composed of thousands of atoms.

In recent years, some progress has been made in the study of polymer systems. The introduction of scaling concepts and renormalization techniques has given a foothold in the study of polymer melts and solutions. The experimental testing of these predictions has been accelerated by the introduction of lasers for light scattering and small angle neutron scattering. Eventually, one would like to
be able to predict the properties of new polymers, mixtures, or solutions, or to design polymers with certain properties.

The study of biological systems is one of enormous complexity.\textsuperscript{13} To avoid a purely phenomenological treatment, and in order to achieve a deeper understanding of the fundamental mechanisms in living systems, one must work with simplified, purified components. Great steps have been taken in isolating particular agents from living cells,\textsuperscript{14} but one must not lose sight of the fact that the functioning of a living organism is due to the interplay of complex macromolecules in solvents.\textsuperscript{15} The key to the origin of many diseases and disorders will come only with an understanding of the microscopic nature of cellular function.\textsuperscript{16}

B. The study of macromolecular systems.

When one studies macromolecular systems, two general categories of parameters are of interest.\textsuperscript{17} The essential difference between these two categories is the range of space and time over which quantities are averaged. Equilibrium or static quantities are the long-time-average, large-distance-scale properties. The classical thermodynamic quantities, such as specific heat, are equilibrium quantities. When these quantities are calculated for a system, one must sum over all accessible states of the system; this summing makes the resulting quantities less sensitive to the microscopic details of the system and, thus, effectively removes some information about the microscopic details of the system. Transport or dynamic quantities are short-time-average, small-distance-scale properties related to the time dependent correlation functions for position, velocity or force.\textsuperscript{18} The
calculation of these quantities give results which may depend explicitly on the microscopic details of the system.

A wide variety of techniques are commonly used to study macromolecular systems. Computer simulations are becoming a useful theoretical tool, while several experimental techniques have been used with success in recent years.

Complex molecular systems are being successfully modeled using well established "molecular dynamics" techniques.\textsuperscript{19-25} The simulations start\textsuperscript{26} with a set of atomic coordinates as determined from X-ray crystallography, for example, and a model of the potential energy surface for motion near the crystallographic coordinates. A set of velocities is chosen from a Maxwellian distribution at low temperature, and the system is allowed to equilibrate for a few picoseconds by numerically integrating the equations of motion. Successively higher temperature distributions are used in warming the molecule to the desired simulation temperature. Once this molecule is equilibrated, the Newtonian equations of motion are used to follow the positions and velocities of the atoms for a given length of time, typically on the order of a few hundred picoseconds. One may then calculate quantities of interest such as the mean-square vibrational amplitude, or the mobility of certain segments or groups. Limitations do exist for this method. One is constrained to simulate molecules with well characterized coordinates and force constants. Currently it is prohibitively expensive to examine more than a few hundred picoseconds of motion because of computer costs. Thus, rare events like aromatic ring flips cannot be observed without preparing the system in a special way.\textsuperscript{27}
Other computer techniques are useful. Full blown quantum mechanical calculations may be done using self consistent solutions to Schrödinger's equations. However, the large number of atoms in macromolecules makes this technique impractical. Monte Carlo or stochastic methods may be used when the group or segment of interest may be considered to be in contact with a heat bath which represents the influence of the rest of the molecule or the solvent. For example, aromatic ring flip rates have been computed in this manner.\(^ {28}\)

Many experimental techniques have been used in the study of macromolecular systems. Here we will give a brief sketch of some relevant techniques and references to the literature.

Thermodynamic measurements allow the measurement of static quantities. One may be able to estimate the static pair-pair correlation function in a system through measurements of the equation of state,\(^ {29}\) for example. Or one may be able to estimate molecular dimensions by measuring intrinsic viscosity or phase transition temperatures.\(^ {30}\)

With small angle neutron scattering, one is able to determine the Fourier components of the density fluctuations with spatial resolution down to about 10 angstroms. The observed signal is proportional to the difference in scattering length per unit volume between the solvent and the solute. This allows one to vary the "contrast" of a solution by isotope substitution and determine quantities such as the radius of gyration for specific nuclei in a molecule.\(^ {12,31}\)

Light scattering is very similar to small angle neutron scattering except that the interaction is electronic and not nuclear, and the wavelength of the incident radiation is much larger. Light can be
scattered elastically or inelastically from a sample. Elastic light scattering is useful for obtaining static quantities such as molecular weights and sizes.\textsuperscript{32} Inelastic scattering is useful for measuring dynamic information such as diffusion coefficients (by quasi-elastic or Rayleigh scattering),\textsuperscript{33-34} vibrational modes (by Raman scattering),\textsuperscript{35} or coherent density fluctuations (Brillouin scattering).\textsuperscript{35} The spatial resolution for a light scattering experiment is of the order of 1000 angstroms, which may be too large to see the microscopic details of interest.\textsuperscript{36-37}

Neutron and X-ray diffraction may be used in structural studies of materials. The scattered particles are collected as a function of scattering angle and are not energy analyzed. The scattering intensity is characterized by Bragg peaks in the case of crystalline (single crystal or powder) materials\textsuperscript{38} or oscillatory patterns in the case of molecular liquids.\textsuperscript{39-40} These spectra are the result of coherent scattering; the incoherent contribution is eliminated by isotopic substitution or theoretical subtraction. The information from this type of scattering is generally interpreted structurally, and thus, the molecules have come to be viewed as static structures. Recently, however, a new appreciation of the dynamic nature of macromolecules has arisen,\textsuperscript{41-43} and one now may use X-ray diffraction to study the atomic dynamics in macromolecules.

Nuclear magnetic resonance (NMR) is a well established method of studying the dynamics of nuclei with non-zero nuclear magnetic moments.\textsuperscript{44} Hydrogen is commonly used as the resonant nucleus because of its large natural abundance and large gyromagnetic ratio. The local magnetic environment of a nuclear magnetic moment causes relaxation of
the moment to equilibrium along the externally applied magnetic field (characterized by the time constant $T_1$) and transverse to the externally applied magnetic field (characterized by the time constant $T_2$). The relaxation rate is thus characteristic of the motion of magnetic neighbors. Diffusion coefficients are easily measured using NMR, as well as features which are characteristic of the electronic environment. The time scale of motion observed in an NMR experiment varies from $10^{-3}$ second in a diffusion experiment to $10^{-9}$ or less in a relaxation rate experiment.

Mössbauer spectroscopy exploits the resonant absorption of a gamma ray by a bound nucleus to detect the effect of the chemical environment on the nuclear levels of an absorber. Nuclei used in these experiments are very heavy, such as $^{57}$Fe or $^{61}$Ni so one must have a macromolecular system which can chemically accommodate iron or nickel. One biological molecule which is easily obtained and which has a naturally occurring iron atom is myoglobin. Experimenters have used the Mössbauer effect to examine the short time motion of the iron atom covalently bonded to the heme group in single crystal metmyoglobin.

Finally, quasi-elastic neutron scattering (QNS) can be used to study the dynamics of molecular motion through the Doppler broadening of the energy profile of the incident low energy neutron beam. The primary scattering nucleus used is hydrogen since it is the strongest, most abundant, biologically relevant scatterer. The cross section of hydrogen is mostly incoherent (98%) and so large that, to a good approximation, scattering by other nuclei may be ignored. This is helpful because the scattering laws for incoherent scattering are easier to model in liquids than the coherent scattering laws.
time scale of motion which influences the neutron spectrum varies from $10^{-12}$ to $10^{-6}$ seconds. Also a spatial resolution from 0.1 to 10 angstroms is easily attainable, which is in the range of interest for studying atomic motion.

These are some experimental techniques that have yielded information about the microscopic dynamics in macromolecular systems.

C. The work to be presented in this thesis.

The goal of this work is to examine the dynamics of hydrogen motion in some macromolecular solutions and compounds. The work which predates our measurements will now be discussed and summarized.

Computer simulations have been carried out using molecular dynamics techniques on several large molecules in vacuum and in solutions. These simulations show that the short time motion of protein constituents depends on the local environment in a characteristic way. For example, the mean-square vibrational amplitude of main-chain carbon atoms is larger when in contact with external solvent molecules or the external vacuum than when confined to the interior of the protein.

The motion of individual heavy atoms was measured by Frauenfelder, et al. using X-ray crystallographic methods. Frauenfelder used crystalline metmyoglobin (ferric myoglobin with $H_2O$ at the active site), and he found that there is indeed a correlation between the position of an atom in the protein and its mean-square vibrational amplitude.

Parak, et al. obtained results similar to Frauenfelder's when Mössbauer spectroscopy was used to examine the motion of iron bound to the heme group in metmyoglobin. In addition, they detected a
disappearance of some contribution to the vibrational amplitude as the temperature was lowered below 210K. This disappearance was not associated with any macroscopic phase transition, nor was it associated with phonon-like intermolecular vibrations. However, the additional motion above 210K was asserted to be a characteristic of molecules which are experiencing intramolecular fluctuations between conformational substates.

Our experiment is designed to see if this type of temperature dependence is found in the high frequency motions of hydrogen atoms associated with proteins and polymers.

The high frequency vibrational motion of hydrogen in molecular systems is manifested as a reduction of the intensity of the quasi-elastic neutron spectrum as a function of increasing momentum transfer \(Q\).\(^{67-68}\) This intensity may be extracted, in the usual manner,\(^{69-70}\) by scanning the quasi-elastic energy peak at high energy-resolution and fixed \(Q\), deconvolving the spectrum and the spectrometer resolution function, and numerically finding the area under the quasi-elastic peak. This is repeated for a set of \(Q\) values, and a fit of the intensity to \(I = I_0 \exp(-\langle u^2 \rangle Q^2/3)\) gives \(\langle u^2 \rangle\), the mean-square vibrational amplitude of the scattering nuclei.

In principle, the intensity and mean-square vibrational amplitude determined in this manner is reliable. Also, a careful analysis of the line shape can reveal the diffusive dynamics of the scattering nuclei. However, there are drawbacks to this technique. The counting rate is low, since the energy window is narrow and cold neutrons must be used, so a correspondingly long time must be spent counting. This means that biological systems which may deteriorate are not likely to be amenable
to this method (for an exception see reference 60). And since neutron beam time is precious at the national labs, one may not have enough time to collect enough data to draw valid conclusions. In addition, the accessible range of energy transfer and momentum transfer available on a spectrometer is smaller when the energy resolution is increased. Finally, one must have a reasonable model for the dynamic line broadening which occurs. This is not difficult in "simple" systems like pure H₂O, but in complex macromolecular systems, the motion is quite complicated and the line width function may be too complex to fit the data with any confidence given a limited range scan of energy-Q space.

An alternative to the above high resolution method is to use a low energy-resolution detector centered at zero energy transfer and effectively integrate the quasi-elastic energy peak without doing an energy scan. The count rate is increased, over the above method, because the energy resolution is of the order of the width of the quasi-elastic linewidth and because we are able to use thermal neutrons which have a higher flux at the High Flux Isotope Reactor (HFIR). We are able to cover a Q range that is twice as large as that in the high energy-resolution experiments, with smaller Q-step size. There are several difficulties with this method. It is impossible to extract diffusional characteristics from the data. This is because the lineshape is not measured. Also, correcting for the inelastic background, correcting for the non-ideal energy resolution, and subtracting the solvent contribution to the intensity are non-trivial problems. These difficulties will be addressed in the following pages.
We have examined two polymeric samples with these techniques. We collected high energy-resolution QNS data on poly(ethylene oxide), or Polyox, in the hydrogenated and deuterated forms in D$_2$O and H$_2$O. This data was taken for 5 or 6 Q-values at room temperature (See Table 1). Polyox is a water-soluble, linear chain polymer with (-CH$_2$-CH$_2$-O-) as the monomer. Many chemical and physical studies have been conducted on Polyox, including QNS, NMR, electron spin resonance, and light scattering. Since it is available in several commercially produced molecular weights, we have chosen to look at both low and high molecular weight species.

We collected integrated intensity or low energy-resolution QNS data on Polyox (hydrogenated only) and trypsin powder and their D$_2$O solutions as a function of temperature between 75K and 300K (See Table 1). Trypsin is a digestive proteolytic enzyme (it catalyzes the hydrolysis of a peptide bond) which is secreted by the pancreas as the precursor trypsinogen into the duodenal tract of many species of animals. The literature on trypsin is extensive, and includes recent molecular dynamics computer simulations.
TABLE 1. A compilation of the experiments reported on in this thesis.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample (Concentration in wt %)</th>
<th>Temperature</th>
<th>Q Range (Å⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Resolution</td>
<td>Deuterated Polyox(^a)</td>
<td>Room Temp</td>
<td>0.5, 0.7, 1.0, 1.2, 1.5, 1.9</td>
</tr>
<tr>
<td></td>
<td>in H₂O : 5%, 10%, 20%, 38%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deuterated Polyox(^a)</td>
<td>Room Temp</td>
<td>0.7, 1.5</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polyox (WSR-205)(^b)</td>
<td>Room Temp</td>
<td>0.5, 0.7, 1.0, 1.2, 1.5, 1.9</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrated QNS</td>
<td>Empty Chamber</td>
<td>300-100K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>300-250K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>D₂O</td>
<td>300-150K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>Polyox (WSR-205)(^b)</td>
<td>300-75K</td>
<td>0.5 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 18.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polyox (8000)(^c)</td>
<td>300-125K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 18.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsin (Raw)(^d)</td>
<td>300-75K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsin (Treated)(^e)</td>
<td>300-75K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
<tr>
<td></td>
<td>in D₂O : 20%</td>
<td></td>
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<tr>
<td></td>
<td>Polyox (WSR-205)(^b)</td>
<td>300-75K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
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<tr>
<td></td>
<td>Powder</td>
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<tr>
<td></td>
<td>Trypsin (Raw)(^d)</td>
<td>300-75K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
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<tr>
<td></td>
<td>Powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsin (Treated)(^e)</td>
<td>300-75K</td>
<td>0.1 to 4.1 in 0.1 steps</td>
</tr>
</tbody>
</table>

\(^a\) Deuterated Polyox is product MD-2747 from Merck and Co., Rahway, N.J., Lot 130-H. Molecular weight is approximately 600,000 daltons.

\(^b\) Polyox (WSR-205) is from Union Carbide Corp., New York, Lot 1113. Molecular weight is approximately 8400 daltons.

\(^c\) Polyox (8000) is Polysol E-8000 from Dow Chemical Co., USA, Lot TB850711 FIH. Molecular weight is approximately 8400 daltons.

\(^d\) Trypsin is product T-2395 from Sigma Chemical Co., St. Louis, MO., Lot 65F-8190. "Raw" means straight from the bottle. Molecular weight is approximately 23,800 daltons.

\(^e\) Same product as (d). "Treated" means D₂O exchange/freeze dry cycle has been used.
EXPERIMENTAL DETAILS

A. The neutron spectrometer

The neutron scattering experiments were done at the High Flux Isotope Reactor (HFIR) at Oak Ridge National Laboratory (ORNL). The HFIR is a light-water cooled, light-water moderated fission reactor, with a beryllium reflector. The neutrons are produced by the fission of $^{235}\text{U}$, which is contained in aluminum-clad plates arranged in cylindrical geometry. The highly energetic neutrons released in the fission collide with the nuclei of the moderator and thus give up kinetic energy. At some distance from the center of the core, the neutrons are in thermal equilibrium with the moderator and the beryllium reflector, and may be considered an ideal gas at the temperature of the moderator (300K). This "thermal" neutron gas may then be removed from the reactor with a beam tube and used in experiments.

The HFIR has four horizontal beam tubes equipped for experiments. The instrument we used in these experiments was a triple-axis spectrometer designated HB-2, and is shown schematically in Figure 1. HB-2 is designed to allow many types of experiments, but of primary interest to us is the ability to make scans of (Q,ω) space at constant Q while varying the neutron energy change, ω. For a constant-Q scan, the incident energy is continuously varied by changing the monochromator angle, $\theta_m$, to select a wavelength according to

$$\lambda_0 = 2d_{hkl} \sin \theta_m$$

where $d_{hkl}$ is the spacing of the lattice planes involved in the coherent Bragg reflection. A variable incident energy is desirable because one is not obliged to correct the final detector count for the energy dependent detector efficiency. In the high
resolution experiments, the beam was made monochromatic by the (002) reflection from pyrolytic graphite\textsuperscript{92} ($d_{\text{002}}=3.3480\text{Å}$); in the low resolution experiments the (101) reflection from beryllium was used ($d_{\text{101}}=1.7326\text{Å}$). The flux at the sample is higher in the low resolution experiments because we used a higher energy incident beam ($E_i=13.8\text{meV}$ or 160K) than in the high resolution experiments ($E_i=3.93\text{meV}$ or 45K).
There are more neutrons at the higher energy since that energy is closer to the peak of the velocity distribution curve for thermal neutrons.

The incident beam was collimated by a series of cadmium coated vertical vanes to a divergence of 20 arc seconds in the high resolution experiments and 30 arc seconds in the low resolution experiments. Higher order monochromator reflections ($\lambda_0/n, n=2,3,...$) were removed by polycrystalline filters$^{93-94}$ (beryllium in the high resolution case and pyrolytic graphite in the low resolution case). The beam was then monitored with a uranium counter so that the number of incident neutrons was constant at each value of $\omega$.

The samples were held in an aluminum sample chamber (Figure 2) with a 1 mm thick aluminum cover plate which was sealed with a 99.99% pure indium wire seal and secured with aluminum screws and nuts. Indium is a strong neutron absorber and was thus virtually opaque to the beam. Aluminum is nearly transparent to thermal neutrons, except when the Bragg condition is met; these strong Bragg peaks are easily avoided or removed from the spectrum. In the low resolution experiments, the sample holder was attached to a refrigerated "cold finger" and sealed in an evacuated ($10^{-3}$ to $10^{-4}$ torr) aluminum cryostat for temperature control. For the high resolution experiments, the sample holder was mounted in the open, at room temperature.

After the neutrons are scattered, the beam was again collimated with vertical cadmium coated vanes to a divergence of 20 arc seconds in the high resolution experiments and 40 arc seconds in the low resolution experiments. The beam was then energy analyzed at fixed
Figure 2. The aluminum sample chamber.

final energy by a Bragg reflection from the (002) plane of pyrolytic graphite. The neutrons were then counted with a $^3$He detector.$^{95}$

The energy resolution of the entire spectrometer depends on the monochromator mosaic, the analyzer mosaic, and the vertical and horizontal divergence of the beam.$^{96}$ The resolution was measured at the time of each experiment by measuring the incoherent, elastic scattering from a vanadium sample. The resolution function was found to be satisfactorily fit with a Gaussian function of $\omega$ whose full width at
TABLE 2. Spectrometer parameters.

<table>
<thead>
<tr>
<th>Date</th>
<th>$E_r$ (meV)</th>
<th>Monochromator</th>
<th>Filter</th>
<th>Analyzer</th>
<th>Resolution (FWHM of Gaussian fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 1983</td>
<td>3.928</td>
<td>PG$^a$(002)</td>
<td>Be$^b$</td>
<td>PG$^a$(002)</td>
<td>$0.02276 \pm 0.0003$ THz, $94 \pm 1$ µeV</td>
</tr>
<tr>
<td></td>
<td>3.928</td>
<td>PG$^a$(002)</td>
<td>Be$^b$</td>
<td>PG$^a$(002)</td>
<td>$0.01918 \pm 0.0008$ THz, $79 \pm 3$ µeV</td>
</tr>
<tr>
<td>Jan. 1986</td>
<td>13.8</td>
<td>Be$^c$(101)</td>
<td>PG$^a$</td>
<td>PG$^a$(002)</td>
<td>$0.1603 \pm 0.004$ THz, $663 \pm 18$ µeV</td>
</tr>
</tbody>
</table>

$^a$pyrolytic graphite.
$^b$polycrystalline Beryllium.
$^c$single crystal Beryllium.

The half maximum (FWHM) is given in Table 2. Table 2 summarizes the spectrometer parameters for each experiment.

B. Sample preparation.

The Polyox, in all cases, was used as supplied by the manufacturer with no further treatment, except drying. Since Polyox is quite hygroscopic, we dried it by placing it in a desiccator over CaSO$_4$ (Drierite) under slight vacuum for more than 24 hours. The appropriate amounts of Polyox and solvent (distilled, deionized H$_2$O or 99.8% pure D$_2$O) were placed in a glass or plastic jar, sealed, and agitated by hand. For the high molecular weight Polyox (WSR-205) at 20% concentration, it took roughly 24 hours of occasional agitation to mix thoroughly. All of the other concentrations were well mixed in a few minutes. The solutions were kept cold in a refrigerator or on ice for storage or transportation to inhibit bacterial growth. When the
temperature was varied in the course of an experiment, we started at 300K and reduced it in 25K steps. Sometimes we repeated the data collection at a higher temperature after being at a low temperatures to see if there was any change in the spectrum. No significant change was apparent.

Trypsin has covalently bonded hydrogens which can exchange readily with solvent hydrogens. Since we were interested in using D₂O as a solvent, and we were interested only in scattering from trypsin hydrogens, we exchanged the labile hydrogens with deuterons using the following process. Trypsin powder was mixed with D₂O in a sealed flask at about 6 wt% concentration. The flask was left for at least 15 hours in a refrigerator at 4 to 6°C. This solution was then freeze dried. The resulting trypsin is called "treated" trypsin throughout this thesis. We have also done experiments with trypsin which was not treated in this manner. This is called "raw" trypsin throughout this thesis.

The appropriate amount of trypsin was mixed with D₂O which was pH adjusted using DCl to a pH between 4 and 5. The use of an acidic pH was intended to inhibit any enzymatic activity since the trypsin is proteolytic and, if not inhibited, will become inactive through autodigestion. Once a sample was prepared, it was placed on the sample support in the cryostat and cooled to 275K as quickly as possible. The time between adding the D₂O/DCl solution to the trypsin powder and reaching 275K was always less than one hour. The temperature sequence for the trypsin was different than that for the Polyox. The trypsin solutions were cooled from the initial 275K to 75K in 25K steps, then warmed to 275K, then warmed to 300K, then cooled to
275K, again. No appreciable differences were found in any of the 275K spectra.

We wanted to make sure that the trypsin was not damaged by the freeze drying and temperature cycling, so we performed an enzymatic assay on some of the excess trypsin. We followed the assay procedure used by Sigma Chemical Company to determine the activity in the same manner as the manufacturer. This involves observing the time rate of change of the absorbance of a sample at 253 nm as trypsin hydrolyzes the substrate α-N-Benzoyl-L-Arganine Ethyl Ester (BAEE) at pH 7.6 at 25°C in a reaction volume of 3.2 ml. We performed the assay on five samples: raw trypsin powder, treated trypsin powder, freeze cycled raw trypsin in D₂O/DCl, freeze cycled treated trypsin in D₂O/DCl, and freeze cycled raw trypsin in H₂O. The freeze cycle mentioned here is meant to simulate the treatment the actual scattering samples received. First, the samples were prepared at 20% concentration. Second, they were allowed to sit at room temperature for about one hour. Third, they were frozen in a conventional freezer for at least 24 hours at between -5 to -20°C. Fourth, they were allowed to thaw and sit at room temperature for at least one-half hour. Finally, they were freeze dried. The results of the assay are shown in Table 3. All of the activities measured are in the range expected for this trypsin sample, and, thus, show that the trypsin was probably intact throughout these experiments.

The weights of the prepared sample chambers were measured before and after an experiment in order to see if any significant sample loss occurred. In the room temperature experiments, the weight loss was always less than one percent of the estimated sample weight. However,
TABLE 3. Results of enzymatic assay of trypsin samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity (BAEE units/ mg solid)</th>
<th>Number of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin (Jar label)</td>
<td>13,000</td>
<td>0</td>
</tr>
<tr>
<td>Raw powder</td>
<td>13,500 ± 800</td>
<td>3</td>
</tr>
<tr>
<td>Treated powder</td>
<td>11,500 ± 600</td>
<td>5</td>
</tr>
<tr>
<td>Freeze cycled raw trypsin in D₂O/DCl</td>
<td>12,400 ± 600</td>
<td>4</td>
</tr>
<tr>
<td>Freeze cycled treated trypsin in D₂O/DCl</td>
<td>13,600 ± 500</td>
<td>4</td>
</tr>
<tr>
<td>Freeze cycled raw trypsin in H₂O</td>
<td>12,700</td>
<td>2</td>
</tr>
</tbody>
</table>

in the temperature controlled experiments, the weight loss sometimes was as large as three percent of the estimated sample weight. This may be explained by noting that the sample was under vacuum in the cryostat, and that during the sealing of the sample chamber, some solution may be trapped on the outside of the indium seal between the chamber and the cover. The trapped solution is quickly dried in the first few minutes under vacuum, and, thus, there is probably little change in sample volume during the actual scattering experiment. Some dry residue was observed on the outside of some of the chambers, but no significant amount of sample leaked out.
THEORETICAL BACKGROUND

A. General results.

A thermal neutron of energy $E_1 = mv_1^2/2$ and wavevector $\mathbf{k}_1$ incident on a sample may be scattered by a nucleus in the sample with a change in energy to $E_2 = mv_2^2/2$ and a change in wavevector to $\mathbf{k}_2$. The partial differential cross section for this type of scattering is given by: 56-62, 100-105

$$\frac{d^2 \sigma}{d\Omega dE_2} = \frac{N}{4\pi} \frac{k_2}{k_1} \left\{ \sigma_c S(Q, \omega) + \sigma_i S(Q, \omega) \right\}$$  \hspace{1cm} (1)

$d^2 \sigma$ = Probability that an incident neutron with energy $E_1$ and wavevector $\mathbf{k}_1$ is scattered into the solid angle element $d\Omega$ with final energy between $E_2$ and $E_2 + dE_2$, with wavevector $\mathbf{k}_2 = \mathbf{k}_1 - \mathbf{Q}$.

with:

$$\hbar \omega = E_1 - E_2 = \frac{m}{2} \left( v_1^2 - v_2^2 \right) = \frac{\hbar^2}{2m} \left( k_1^2 - k_2^2 \right)$$ \hspace{1cm} (2)

= Energy lost by the neutron

$\mathbf{Q} = \mathbf{k}_1 - \mathbf{k}_2 = $ Scattering vector \hspace{1cm} (3)

$N$ = The number of identical nuclei in the sample.

$\sigma_c, \sigma_i$ = The coherent and incoherent bound cross sections,
respectively.

\[ S(\mathbf{Q}, \omega), \quad S_1(\mathbf{Q}, \omega) = \text{The coherent and incoherent "scattering laws",} \]

respectively.

The scattering laws may be evaluated in the Born approximation in terms of correlation functions which are characteristic of the scattering nuclei. We introduce the van Hove correlation functions:

\[
G(\mathbf{r}, t) = N^{-1} \sum_{i,j} \left< \int \mathcal{d}\mathbf{r}' \, \delta(\mathbf{r} + \mathbf{r}_i(0) - \mathbf{r}') \delta(\mathbf{r}' - \mathbf{r}_j(t)) \right> 
\]

(4)

\[
G_s(\mathbf{r}, t) = N^{-1} \sum_{i} \left< \int \mathcal{d}\mathbf{r}' \, \delta(\mathbf{r} + \mathbf{r}_i(0) - \mathbf{r}') \delta(\mathbf{r}' - \mathbf{r}_i(t)) \right> 
\]

(5)

where \(<\cdots> = \sum_{\lambda} \frac{\exp[-\beta E_\lambda]}{Z} <\lambda|\cdots|\lambda>\) is called a "thermal average" and the average \(N^{-1} \sum_{i} \) may be dropped if all the nuclei are identical. \(S(\mathbf{Q}, \omega)\) and \(S_1(\mathbf{Q}, \omega)\) are then given by:

\[
S(\mathbf{Q}, \omega) = (2\pi \hbar)^{-1} \int \mathcal{d}t \int \mathcal{d}\mathbf{r} \, e^{i \mathbf{Q} \cdot \mathbf{r}} G(\mathbf{r}, t) 
\]

(6)

\[
S_1(\mathbf{Q}, \omega) = (2\pi \hbar)^{-1} \int \mathcal{d}t \int \mathcal{d}\mathbf{r} \, e^{i \mathbf{Q} \cdot \mathbf{r}} G_s(\mathbf{r}, t) 
\]

(7)

It is sometimes useful to deal with the intermediate scattering functions given by:

\[
I(\mathbf{Q}, t) = \int \mathcal{d}\mathbf{r} \, e^{i \mathbf{Q} \cdot \mathbf{r}} G(\mathbf{r}, t) 
\]

(8)

\[
I_s(\mathbf{Q}, t) = \int \mathcal{d}\mathbf{r} \, e^{i \mathbf{Q} \cdot \mathbf{r}} G_s(\mathbf{r}, t) 
\]

(9)
The van Hove correlation functions are complex, quantum mechanical functions, in general. However, when the classical approximation is valid, i.e.:

\[ |\text{me}| \ll k_BT/2 \quad \text{and} \quad \frac{(\text{RQ})^2}{2M} \ll k_BT/2 \quad (10) \]

then the van Hove correlation functions have a simple physical interpretation. The \( C^{	ext{cl}}(\mathbf{r},t)d^2 \mathbf{r} \) is the probability of finding any atom in the volume element \( d^2 \mathbf{r} \) at \((\mathbf{r}+\mathbf{r}_0, t)\) given that a particular atom was at \((\mathbf{r}_0, t=0)\) averaged over all of the initial sites, \( \mathbf{r}_0 \), in the volume. Similarly, \( C^{	ext{cl}}(\mathbf{r},t)d^2 \mathbf{r} \) is the probability of finding the same atom in the volume element \( d^2 \mathbf{r} \) at \((\mathbf{r}+\mathbf{r}_0, t)\) given that that particular atom was at \((\mathbf{r}_0, t=0)\) averaged over all of the initial sites, \( \mathbf{r}_0 \), in the volume.

As stated in the Introduction, the scattering from hydrogen containing systems is dominated by the incoherent scattering from hydrogen. So, we will not discuss the coherent scattering from here on except when discussing the scattering from the D\(_2\)O samples, or in connection with Bragg peaks. Also, we will consider only classical calculations of the scattering laws, so we can drop the superpostscript "cl" from the notation.

B. Separation of different contributions to the motion.

In the classical limit, then, the quantum mechanical operators in equation (5) commute and we have:

\[ G^\text{cl} (\mathbf{r},t) = N^{-1} \sum_{i=1}^{N} \langle \delta(\mathbf{r} + \mathbf{r}_i(t) - \mathbf{r}_i(0)) \rangle \quad (11) \]
Then the intermediate scattering function is:

\[ I_s(\mathbf{Q}, t) = \int d\mathbf{r} e^{i\mathbf{Q} \cdot \mathbf{r}} G_s(\mathbf{r}, t) \]

\[ = N^{-1} \sum_{i=1}^{N} < \int d\mathbf{r} e^{i\mathbf{Q} \cdot \mathbf{r}} \delta(\mathbf{r} + \mathbf{r}_i(0) - \mathbf{r}_i(t)) > \]

\[ = N^{-1} \sum_{i=1}^{N} < e^{i\mathbf{Q} \cdot [\mathbf{R}_i(t) - \mathbf{r}_i(0)]} > \quad (12) \]

We may be able to take the position of the \( i \)th scattering nucleus as: \(^{107-109}\)

\[ \mathbf{r}_i(t) = \mathbf{a}_i(t) + \mathbf{R}_i(t) + \mathbf{u}_i(t) \quad (13) \]

where \( \mathbf{a}_i(t) \) is the vector from the origin to the center of mass of the molecule containing the \( i \)th nucleus, \( \mathbf{R}_i(t) \) is the vector from the molecular center of mass to the average position of the \( i \)th nucleus, and \( \mathbf{u}_i(t) \) is the displacement of the \( i \)th nucleus relative to the average position of the atom. The component \( \mathbf{u}_i(t) \) may be taken as the vibrational amplitude of the \( i \)th nucleus, for example. Using equation (13), equation (12) becomes:

\[ I_s(\mathbf{Q}, t) = N^{-1} \sum_{i=1}^{N} < e^{i\mathbf{Q} \cdot (\mathbf{a}_i(t) - \mathbf{a}_i(0))} e^{i\mathbf{Q} \cdot (\mathbf{R}_i(t) - \mathbf{R}_i(0))} e^{i\mathbf{Q} \cdot (\mathbf{u}_i(t) - \mathbf{u}_i(0))} > \quad (14) \]

If we may presume that the different types of motion considered (for example: center of mass translation, molecular rotation or conformation fluctuation, or atomic vibration) are independent of one another, then equation (14) becomes:
\[ I_s(\hat{Q}, t) = N^{-1} \sum_{i} \langle e^{i\hat{Q} \cdot (\hat{R}_i(t) - \hat{R}_i(0))} \rangle \langle e^{i\hat{Q} \cdot (\hat{\Omega}_i(t) - \hat{\Omega}_i(0))} \rangle \]

\[ = N^{-1} \sum_{i} I_s^{ai}(\hat{Q}, t) I_s^{\Omega i}(\hat{Q}, t) I_s^{\Omega i}(\hat{Q}, t) \]  

If we assume, further, that all of the scattering nuclei in the sample are equivalent or that we may replace all of the scattering centers with an "average" scattering center, we get:

\[ I_s(\hat{Q}, t) = I_s^{q}(\hat{Q}, t) I_s^{R}(\hat{Q}, t) I_s^{\Omega}(\hat{Q}, t) \]  

Then the convolution property of Fourier transforms gives, using equation (7) and (17):

\[ S_i(\hat{Q}, \omega) = n^2 S_i^q(\hat{Q}, \omega) * S_i^R(\hat{Q}, \omega) * S_i^{\Omega}(\hat{Q}, \omega) \]  

where * means a convolution in \( \omega \). Thus, if we are able to assert that:

1) all of the scattering centers in a sample are equivalent or may be replaced by an "average" scattering center, and 2) the overall motion of an atom may be resolved into uncorrelated components, then we get the total incoherent scattering law by convoluting all of the scattering laws for each individual component of the motion.

C. The Gaussian approximation.

For the systems in which \( G_s(r, t) \) is calculable exactly, such as an ideal gas, a harmonically bound nucleus or in the case of Brownian diffusion, the form is found to be Gaussian in \( r \). Thus, a common
starting point for calculating a scattering law is to take a normalized Gaussian function of \( r \), with a full width at half maximum squared equal to \((8\ln 2)w(t)\), which is an explicit function of time. The normalization for this function may be determined from equation (11) and is:

\[
\int dr^r G_s(r^r, t) = 1. \tag{19}
\]

Thus, the Gaussian approximation involves assuming:110-112

\[
G_s(r^r, t) = \left(2\pi w(t)\right)^{-3/2} \exp\left[-r^2/2w(t)\right]. \tag{20}
\]

Using equation (9), we obtain:

\[
I_s(q, t) = \exp\left[- \frac{q^2 w(t)}{2}\right]. \tag{21}
\]

The physical interpretation of \( w(t) \) becomes clear when we calculate the mean-square displacement:

\[
<r^2> = \langle [\dot{r}(t) - \dot{r}(0)]^2 \rangle = \int dr^r r^2 G_s(r^r, t)
\]

\[
<r^2> = 3w(t) \tag{22}
\]

Also, \( w(t) \) is related to the velocity autocorrelation function through:
\[ w(t) = \frac{2}{3} \int_0^t dt' \langle t-t' \rangle \langle \hat{\Phi}(0) \cdot \hat{\Phi}(t') \rangle. \quad (23) \]

As an example, consider a scatterer which is associated with a site which, for the moment, may be considered fixed in space. The atom vibrates around this site with the normalized probability distribution:\textsuperscript{113}

\[ \rho(\vec{r}) = \left(2\pi\langle u^2\rangle/3\right)^{-3/2} \exp[-3r^2/2\langle u^2\rangle]. \quad (24) \]

With this distribution, the mean-square position is:

\[ \int d^3 r' r^2 \rho(\vec{r}) = \langle u^2 \rangle = 3\langle x^2 \rangle = 3\langle y^2 \rangle = 3\langle z^2 \rangle. \quad (25) \]

This quantity, \( \langle u^2 \rangle \), is generally quoted as the squared amplitude of the thermal cloud associated with a vibrating atom. Now, for large times, \( G_S(\vec{r}, t) \) may be shown to be:

\[
\lim_{t \to \infty} G_S(\vec{r}, t) = G_S(\vec{r})
= \int d^3 r'' \rho(\vec{r}' - \vec{r}) \rho(\vec{r}'') \quad (26)
\]

Using equation (24) in equation (26) gives:

\[ G_S(\vec{r}) = \left(4\pi\langle u^2\rangle/3\right)^{-3/2} \exp[-3r^2/4\langle u^2\rangle], \quad (27) \]

which is Gaussian in \( r \) and has a width function:
\[ w(t) = \frac{2\langle u^2 \rangle}{3} \quad \text{(vibrating atom)} \] (28)

or in terms of the mean-square displacement of equation (22), the size of the thermal cloud is:

\[ \langle u^2 \rangle = \frac{\langle r^2 \rangle}{2} \] (29)

From equation (21) we get:

\[ I_S(\mathbf{Q}, t) = \exp\left[-\frac{Q^2 \langle u^2 \rangle}{3}\right] \quad \text{(vibrating atom)} \] (30)

and then the incoherent scattering law is:

\[ S_I(\mathbf{Q}, \omega) = \frac{(2\pi\hbar)^{-1}}{\hbar} \int dt \ e^{-i\omega t} \ I_S(\mathbf{Q}, t) \] (31)

\[ = \frac{(2\pi\hbar)^{-1}}{\hbar} \int dt \ e^{-i\omega t} \ e^{-Q^2 \langle u^2 \rangle/3} \] (32)

\[ S_I(\mathbf{Q}, \omega) = e^{-Q^2 \langle u^2 \rangle/3} \delta(\hbar\omega) \quad \text{(vibrating atom)} \] (33)

\[ = e^{-Q^2 \langle r^2 \rangle/6} \delta(\hbar\omega) \quad \text{(vibrating atom)} \] (34)

The form of equation (33) gives a decrease in intensity with increasing \( Q \) and this is usually called the Debye-Waller factor. This scattering law for a vibrating atom at a fixed site may then be combined with scattering laws for other types of motion according to equation (18) to obtain the total incoherent scattering law. For
example, we could calculate the scattering law for Brownian diffusion of the atom and combine it with equation (33) or (34) to obtain the scattering law for a diffusing, vibrating atom.

We may find the scattering law for Brownian diffusion by considering an equation of motion for a particle subject to a random force whose origin is a bath of similar particles. The atom experiences a random force, \( \hat{F}(t) \), which, for sufficiently long times, has no correlation with itself or the velocity, and which on the average is zero. That is, for \( t >> 0 \):

\[
\langle \hat{F}(t) \rangle = 0, \tag{35}
\]
\[
\langle \hat{F}(0) \cdot \hat{v}(t) \rangle = 0, \tag{36}
\]
\[
\langle \hat{F}(0) \cdot \hat{F}(t) \rangle = 0. \tag{37}
\]

The equation of motion for a particle of mass \( M \) subject to this random force is called the Langevin equation and is given by:

\[
\frac{M \ddot{v}}{dX} = -\gamma \dot{v}(t) + \hat{F}(t), \tag{38}
\]

where \( \gamma \) is the friction coefficient. This may be integrated to find \( \dot{v}(t) \), and then the average \( \langle \dot{v}(0) \cdot \dot{v}(t) \rangle \) is formed. The result is:

\[
\langle \dot{v}(0) \cdot \dot{v}(t) \rangle = \langle \dot{v}^2(0) \rangle e^{-\gamma |t|}. \tag{39}
\]

From equation (23) we form:
\[ w(t) = \frac{2}{3} \int_0^t dt' (t-t') \langle \mathcal{V}^2(0) \rangle e^{-\gamma |t'|} \]

\[ = \frac{2}{3} \langle \mathcal{V}^2(0) \rangle \gamma^{-2} \left[ \gamma t + e^{-\gamma t} - 1 \right] \quad (41) \]

For \( t << \gamma^{-1} \), \( w(t) \sim t^2 \), which is the width function for an ideal gas atom which does not suffer any collisions. This behavior is expected of any model for short times. For larger times, equation (41) is further reduced by considering the diffusion coefficient defined by:

\[ D = \frac{1}{3} \lim_{t \to \infty} \frac{1}{2t} \langle (\mathcal{R}(t) - \mathcal{R}(0))^2 \rangle \]

\[ = \frac{1}{3} \lim_{t \to \infty} \frac{1}{2t} \langle r^2 \rangle \quad (42) \]

\[ = \frac{1}{3} \lim_{t \to \infty} \frac{1}{2t} 3w(t) \]

\[ = \frac{1}{3} \lim_{t \to \infty} \frac{1}{2t} \int_0^t dt' (t-t') \langle \mathcal{V}(0) \cdot \mathcal{V}(t') \rangle \]

\[ D = \frac{1}{3} \int_0^\infty dt \langle \mathcal{V}(0) \cdot \mathcal{V}(t) \rangle. \quad (45) \]

Using equation (41) in (44), we obtain:

\[ D = [3\gamma]^{-1} \langle \mathcal{V}^2(0) \rangle \]

\[ (47) \]

Then using equation (47) in (41), with \( t > \gamma^{-1} \) we get:

\[ \lim_{t \to \gamma^{-1}} w(t) = w_d(t) \quad (48) \]

\[ w_d(t) = 2D[t - \gamma^{-1}] \quad (49) \]
Ignoring the small time, $\gamma^{-1}$, which represents a delay before the motion becomes truly Brownian, we obtain:

$$I_3(Q,t) = \exp[DQ^2t] \quad \text{(Continuous diffusion)} \quad (50)$$

and:

$$S_1^d(Q,\omega) = (\pi \hbar)^{-1} \frac{DQ^2}{\omega^2 + (DQ^2)^2} \quad \text{(Continuous diffusion)} \quad (51)$$

This scattering law is Lorentzian in $\omega$, with a full-width at half-maximum (FWHM) of:

$$\Gamma(Q) = 2DQ^2 \quad \text{(Continuous diffusion)} \quad (52)$$

and an integral area:

$$\int_{-\infty}^{\infty} d(\hbar\omega) \ S_1^d(Q,\omega) = 1. \quad (53)$$

To get the scattering law for an atom which is vibrating around a site which is slowly diffusing, we use equation (18) to form:

$$S_1^d(Q,\omega) = n \ S_1^y(Q,\omega) * S_1^q(Q,\omega) \quad (54)$$

$$= n \int_{-\infty}^{\infty} S_1^y(Q,\omega') * S_1^q(Q,\omega - \omega') \ d\omega' \quad (55)$$

$$= (\pi \hbar)^{-1} e^{-Q^2<\!\omega^2>/3} \int_{-\infty}^{\infty} d\omega' \frac{DQ^2 \delta(\omega')}{(\omega - \omega')^2 + (DQ^2)^2} \quad (56)$$
\[ S^d(Q, \omega) = (\pi n)^{-1} e^{-Q^2 u^2 / 3} \frac{DQ^2}{\omega^2 + (DQ^2)^2} \]  
(Vibration-diffusion) (57)

This scattering law is also Lorentzian in \( \omega \), with a FWHM given by equation (52), but in contrast to equation (53), the integral area in \( \omega \) is \( \exp[-Q^2 u^2 / 3] \).

Experiments have shown that equation (57) for a vibrating, continuously diffusing atom is inadequate for explaining the quasi-elastic scattering from liquids, water in particular.\(^{116-117}\) The primary experimental contradiction is a leveling off of the linewidth with increasing \( Q^2 \), as opposed to the linewidth given by equation (52) which continually increases with \( Q^2 \). However, the intensity variation with \( Q^2 \) as suggested by the Debye-Waller factor in equation (57) is experimentally observed. This suggests that the atom does spend some time vibrating around quasi-stationary sites in the liquid.

A slightly different approach to calculating the scattering law results in a law which seems to fit the data better without introducing ad hoc parameters.\(^{118-119}\) The atom is considered to be strongly associated with its neighbors so that it stays near a site for a time, \( \tau_0 \), on the average, performing oscillations at that site with a mean-square amplitude \( \langle u^2 \rangle \). The atom then diffuses to a new site with a diffusion constant \( D_1 \) for an average time \( \tau_1 \), and then oscillates around that site. If we take the limit \( \tau_0 \gg \tau_1 \), which means that the atom stays in the oscillating state a long time compared to the jump time between sites, and if we take an average of the jump lengths with a distribution \( P(\ell) - \ell \exp[-\ell / \langle \ell \rangle] \), the scattering law is:
\[ S^d_1(Q, \omega) = (\pi h)^{-1} e^{-Q^2 \langle u^2 \rangle / 3} \frac{\Gamma/2}{\omega^2 + (\Gamma/2)^2} \text{ (Jump diffusion)} \] (58)

with:

\[ \Gamma(Q) = \frac{2Q^2D}{1 + Q^2D\tau_0} \text{ (Jump diffusion)} \] (59)

and:

\[ D = \langle \dot{x}^2 \rangle / 6\tau_0. \] (60)

This scattering law has been applied to the quasi-elastic spectra with some success, in particular for H₂O and some aqueous solutions. The value of D for H₂O at room temperature is found to be 2.4 \times 10^{-5} \text{ cm}^2\text{s}^{-1} and \tau_0 = 1.2 \times 10^{-12} \text{ s}, which agrees well with determinations using other methods. Also, the Debye-Waller factor gives \langle u^2 \rangle / 3 \approx 0.34 \text{ Å}^2, which is reasonable for a water molecule. The leveling off of the linewidth with Q² as predicted in equation (59) is a feature of this model which has been observed in recent experiments on supercooled H₂O at temperatures below 0°C.\(^{120}\) However, at higher temperatures, the data does not completely level off in the Q-ranges studied, although the jump-diffusion model fits the data satisfactorily. Other theoretical models for the large-Q dependence of the linewidth have been proposed,\(^{121}\) but they typically use more free parameters, and there is no compelling evidence that the jump-diffusion model for translational diffusion is inadequate for our purposes. Hence, we will not discuss these other models.
D. Incoherent molecular scattering.

A refinement to the jump-diffusion model applied to molecular liquids is the inclusion of a rotational scattering law. The $\text{H}_2\text{O}$ molecule can be modeled as an isotropic free rotator, for example, by considering the two hydrogens to be confined to a spherical surface of radius $R$ and subject to random torques due to collisions. The protons then randomly diffuse on this surface and the scattering law is:

$$\hbar \, S_{\mathbf{k}}^0(\mathbf{Q}, \omega) = A_0(Q) \delta(\omega) + \sum_{\ell = 1}^{\infty} A_\ell(Q) \frac{\ell(\ell+1)D_R}{[\ell(\ell+1)D_R]^2 + \omega^2}$$  \hspace{1cm} (61)

where $D_R$ is the rotational diffusion constant, and:

$$A_\ell(Q) = (2\ell + 1) \, j_\ell^2(QR),$$  \hspace{1cm} (62)

and $j_\ell$ is the spherical Bessel function of order $\ell$.

The first few terms of equation (61) have been combined with the jump-diffusion model and used to interpret QNS data. For $\text{H}_2\text{O}$, at room temperature, the effect of rotational motion on the linewidth is small, but at lower temperatures, the broadening due to rotational motion is found to be adequately described by equation (61). Rotational scattering laws for other types of motion, such as hindered rotation, have been calculated.

When one considers scattering from molecular systems and uses a separation of the motion into components like center of mass translation, molecular rotation, and atomic vibration, for example, the
scattering laws for rotation or vibration have a characteristic $\delta(\omega)$ energy dependence as in equations (61) and (33). This is a consequence of the nature of the probability density for these motions. Consider again equation (26) for the long time limit of $G_s(\mathbf{r}, t)$, which is:

$$
\lim_{t \to \infty} G_s(\mathbf{r}, t) = G_s(\mathbf{r})
$$

$$
= \int d\mathbf{r}' \rho(\mathbf{r}' - \mathbf{r}) \rho(\mathbf{r}')
$$

(26)

If the scattering nucleus is free to diffuse throughout the volume, $V$, of the sample, then $G_s(\mathbf{r}) \to 1/V$, and then for $V \to 0$, we have $G_s(\mathbf{r}) \to 0$. However, if the nucleus is confined to a finite volume with a probability density which is a function of $\mathbf{r}$, then $G_s(\mathbf{r}) \neq 0$. Then the intermediate scattering function is:

$$
I_s(\mathbf{Q}, t \to \infty) = \int d\mathbf{r} e^{i\mathbf{Q} \cdot \mathbf{r}} G_s(\mathbf{r})
$$

$$
\equiv I_s(\mathbf{Q})
$$

(63)

And the scattering law becomes:

$$
S_1^\omega(\mathbf{Q}, \omega) = (2\pi)^{-1} \int dt e^{-i\omega t} I_s(\mathbf{Q}),
$$

$$
= h^{-1} I_s(\mathbf{Q}) (2\pi)^{-1} \int dt e^{-i\omega t},
$$

$$
= I_s(\mathbf{Q}) \delta(\hbar \omega).
$$

(64)

The dimensionless coefficient $I_s(\mathbf{Q})$ in equation (64) is called the elastic incoherent structure factor (EISF). The EISF is a result
of diffraction due to the spatial distribution of a scattering center. The complete scattering law may be written:

\[ S_1(\vec{q}, \omega) = S_0^r(\vec{q}, \omega) + S_1^i(\vec{q}, \omega), \]

\[ = I_s(\vec{q}) \delta(\omega) + S_1^i(\vec{q}, \omega), \]

where \( S_1^i(\vec{q}, \omega) \) represents the part of the scattering law which does not have the elastic term and which comes from the van Hove correlation function terms which tend to zero for long times. Thus we make the separation:

\[ G_s(\vec{r}, t) = G_s^r(\vec{r}, t) + G_s^i(\vec{r}), \]

where \( G_s^r(\vec{r}, t) \to 0 \) as \( t \to \infty \).

This behavior has been experimentally observed, but the data analysis is not always straightforward. The EISFs are generally bell shaped around \( Q=0 \), as is the Debye-Waller factor. Since these two terms multiply in the final form of the scattering law, one must have very accurate data to distinguish terms like \( \exp[-\alpha^2Q^2] \) from terms like \( j_0(\alpha R)\exp[-\beta^2Q^2] \), for general values of \( \alpha, \beta, \) and \( R \). Also, the scattering law for the translational motion of the molecules must be convoluted with the rotational and vibrational scattering laws, and this broadens the \( \delta(\omega) \) function so that it tends to blend in with the quasi-elastic spectrum. In addition, the finite resolution function also smears out any singularities in the scattering law.
E. Spectrometer resolution functions.

When we collect data on a triple-axis spectrometer, the number of counts at a particular point in \((Q, \omega)\) space is given approximately by the convolution integral\(^{96, 126}\):

\[
I_0(Q, \omega) = \int_{-Q'} \int_{-\omega'}^{\omega'} dQ_0 \ d\omega_0 \ R(Q_0, \omega_0) \ S_i(Q-Q_0, \omega-\omega_0)
\]  

(67)

where \(R(Q, \omega)\) is the resolution function in \((Q, \omega)\) space. For the spectrometers we have used, we have observed that the width of the \(Q\)-resolution function is much smaller than any feature in a constant \(\omega\) scan, except in the case of a Bragg peak. Then, for fixed \(\omega\), we simply average the scattering law over a narrow window of \(Q\) space in which \(S_i(Q, \omega)\) is changing very little. Thus, in the limit of a very narrow window, we take:

\[
R(Q, \omega) = \delta(Q) \ R(\omega)
\]  

(68)

then equation (67) becomes:

\[
I_0(Q, \omega) = \int_{-\omega'}^{\omega'} d\omega_0 \ R(\omega_0) \ S_i(Q, \omega-\omega_0)
\]  

(69)

For the sake of computational convenience, the limits on the integral in equation (69) are usually taken to be \(\pm\).

We have measured \(R(\omega)\) for our experiments by scanning the elastic incoherent scattering peak from vanadium. The form of \(R(\omega)\) is Gaussian in \(\omega\), and thus we take the resolution function to be:
\[ R(\omega) = \frac{1}{K^1/2} e^{-\omega^2/K^2} \] (70)

where the FWHM, \( \Gamma_r \), is:

\[ \Gamma_r = 2\sqrt{\ln 2} K \] (71)

As an example, consider a scattering law of the form of equation (58) where the FWHM, \( \Gamma \), is in general a function of \( Q \). Then the detected signal is given by:

\[ I_0(Q, \omega) = \int_{-\infty}^{\infty} d\omega_0 \frac{1}{K^1/2} e^{-\omega_0^2/K^2} e^{-Q^2<\nu^2>/3} \frac{(\pi h)^{-1} \Gamma/2}{(\omega - \omega_0)^2 + (\Gamma/2)^2} \] (72)

\[ = \frac{1}{hK^3/2} e^{-Q^2<\nu^2>/3} \int_{-\infty}^{\infty} d\omega_0 \frac{\Gamma/2 e^{-\omega_0^2/K^2}}{(\omega - \omega_0)^2 + (\Gamma/2)^2} \] (73)

The integral in equation (73) is proportional to the real part of the complex Voigt function, \( V(z) \), so we get:

\[ I_0(Q, \omega) = \pi^{-1/2} (\hbar K)^{-1} e^{-Q^2<\nu^2>/3} \text{Re} \left[ \frac{V(\omega K + i\Gamma(Q)/2K)}{K} \right] \] (74)

The function \( V(z) \) is related to the error function of complex argument by:

\[ V(z) = e^{-z^2} \text{erfc}(-iz) \] (75)
\begin{align*}
    &= e^{-z^2} \left( 1 - \text{erf}(-iz) \right) \quad (76)
\end{align*}

and:
\begin{equation}
    \text{erf}(z) = 2^{-1/2} \int_0^z e^{-t^2} \, dt \quad (77)
\end{equation}

Two limits of equation (74) are of interest, and it is easiest to evaluate them at \( \omega = 0 \). The first limit we are interested in is for high resolution experiments where the resolution function is vanishingly narrow. Thus, we evaluate equation (74) for the case where \( \Gamma_r \sim K \to 0 \).

\begin{equation}
    \lim_{K \to 0} I_0(Q, 0) = \frac{1}{\pi} e^{-Q^2 \langle u^2 \rangle / 3} \lim_{K \to 0} (\frac{\Gamma}{2\pi})^{-1} \text{Re} \, V(\frac{1}{2\pi}) \quad (78)
\end{equation}

The rightmost limit is:

\begin{equation}
    \lim_{K \to 0} (\frac{\Gamma}{2\pi})^{-1} \text{Re} \, V(\frac{1}{2\pi}) = \lim_{K \to 0} (\frac{\Gamma}{2\pi})^{-1} e^{(\Gamma/2K)^2} \text{erfc}(\Gamma/2K) \quad (79)
\end{equation}

\begin{equation}
    = 2^{-1/2}(\pi \Gamma)^{-1} \left[ 1 - \frac{1}{2(\Gamma/2K)^2} + \frac{3}{4(\Gamma/2K)^4} + \cdots \right] \quad (80)
\end{equation}

So we have:

\begin{equation}
    \lim_{K \to 0} I_0(Q, 0) = \frac{2}{\pi \pi \Gamma} e^{-Q^2 \langle u^2 \rangle / 3} \left[ 1 - \frac{1}{2(\Gamma/2K)^2} + \frac{3}{4(\Gamma/2K)^4} + \cdots \right] \quad (81)
\end{equation}

which is, to first order, the amplitude of the scattering function at \( \omega = 0 \). In fact, the resolution function we have chosen becomes a delta function in the above limit, and hence would reproduce the scattering law for any value of \( \omega \). Thus, in general, to measure the scattering function we want as narrow a resolution function as possible.
The other limit of equation (74) of interest corresponds to our low resolution experiments in which the resolution function is broader than the quasi-elastic peak. Thus we evaluate:

\[
\lim_{K \gg \Gamma} I_0(Q,0) = \pi^{-1/2} (\text{nk})^{-1} e^{-Q^2\langle u^2 \rangle /3} \left[ 1 - \left( \frac{\Gamma}{2K} \right) 2 \pi^{-1/2} + \left( \frac{\Gamma}{2K} \right)^2 - \ldots \right] \tag{82}
\]

which to first order gives the intensity of the quasi-elastic peak defined by:

\[
I_0(Q) = \int_{-\infty}^{\infty} \frac{d^2q_0}{d\omega dE_Z} d\omega \tag{83}
\]

\[
= h \int_{-\infty}^{\infty} S_1(Q,\omega) d\omega \tag{84}
\]

\[
= e^{-Q^2\langle u^2 \rangle /3} \tag{85}
\]

\[
I_0(Q) = \lim_{K \gg \Gamma} I_0(Q,0) \tag{86}
\]

Thus we can find the quasi-elastic intensity, and hence \( \langle u^2 \rangle \), by using a low resolution spectrometer and counting at \( \omega = 0 \) as we scan in \( Q \).

F. The inelastic background.

In addition to quasi-elastic scattering near \( \omega = 0 \) in which we are interested, there is a contribution to the spectrum near \( \omega = 0 \) from the inelastic scattering from the sample. We call this the inelastic background.

In the high resolution experiments, we hold \( Q \) constant and scan the quasi-elastic peak by varying \( \omega \). In this case we find that the inelastic background is well approximated by a simple linear function of \( \omega \):
\[ I^\text{inel}_0(Q=\text{fixed}, \omega) = A + B\omega. \] (87)

Higher order terms could be included, but for the limited range of \( \omega \) that we scan, this expression seems to be adequate.

In the low resolution experiments, where we position our low energy-resolution detector at \( \omega=0 \) for various Q-values, the situation is not quite so simple. In addition to the quasi-elastic contribution to the signal, we also have a contribution given by:

\[ I^\text{inel}_0(Q, \omega=0) = \int_{-\infty}^{\infty} d\omega \ R(\omega) \ S^\text{inel}_1(Q, \omega) \] (88)

where \( R(\omega) \) is the resolution function as in equation (70), and \( S^\text{inel}_1(Q, \omega) \) is the inelastic scattering law near \( \omega=0 \). The calculation of \( S^\text{inel}_1(Q, \omega) \) has not been done for liquid systems; thus, for insight we turn to the relevant calculation for solids. The scattering law for single-phonon scattering in a crystal is given by:

\[ S^\text{inel}_{i,\pm}(Q, \omega) = (2M)^{-1} Q^2 \exp\left[-2W(Q)\right] \frac{Z(\omega)}{\omega} \left[n(\omega) + 1\right] \] (89)

for a single atom per unit cell, cubic lattice, and where \( M \) is the nuclear mass, \( Z(\omega) \) is the normalized density of states for the crystal, \( n(\omega) \) is the Bose factor:

\[ n(\omega) = \left[e^{\frac{\hbar \omega}{kT}} - 1\right]^{-1} \] (90)

and \( \exp\left[-2W(Q)\right] \) is the Debye-Waller factor, which for a harmonic, cubic lattice becomes \( \exp\left[-Q^2\langle u^2 \rangle / 3\right] \). Equation (89) applies for phonon
creation and annihilation with the understanding that \( \omega > 0 \). This scattering law is of the form:

\[
S_{1,1}^{\text{inel}}(Q, \omega) = f_1(Q) \cdot f_2(\omega)
\]  

(91)

so the contributed signal is:

\[
I_0^{\text{inel}}(Q, \omega=0) = f_1(Q) \int_{-\infty}^{\infty} d\omega \, R(\omega) \cdot f_2(\omega)
\]  

(92)

or:

\[
I_0^{\text{inel}}(Q, \omega=0) = A_0 \, Q^2 \exp\left[-Q^2 <u_1^2>/3\right]
\]  

(93)

where \( A_0 \) is the numerical result of the integration in equation (92). The relative importance of this contribution depends mainly on the magnitude of \( A_0 \) relative to other contributions to the scattering.

To estimate the relative contribution to the total scattering by the inelastic background, we examine the ratio:

\[
C = \frac{I_0^{\text{inel}}(Q, \omega=0)}{I_0^{\text{el}}(Q, \omega=0) + I_0^{\text{inel}}(Q, \omega=0)}
\]  

(94)

\[
= \frac{R}{1 + R}
\]  

(95)

where:

\[
R = \frac{I_0^{\text{inel}}(Q, \omega=0)}{I_0^{\text{el}}(Q, \omega=0)}
\]  

(96)

and we will use a Debye solid model for \( I_0^{\text{inel}} \) and the \( I_0^{\text{el}} \) given by
equation (74) for a jump-diffusing liquid like water. \( I_{0}^{\text{q}}(Q, \omega) \) is given by:

\[
I_{0}^{\text{q}}(Q, \omega = 0) = \frac{\pi^{-1/2}}{(nk)^{-1}} e^{-Q^{2} < u_{q}^{2} > /3} \text{Re} \left\{ \frac{\Gamma(Q)}{2k} \right\}.
\]

(97)

Using equations (88) and (89) and taking:

\[
Z(\omega) = \frac{1}{\frac{\omega^{2}V}{2\pi^{2}c^{3}N}}
\]

(98)

for the density of states in a Debye solid, where \( c \) is the speed of sound, we have:

\[
I_{0}^{\text{inel}}(Q, \omega = 0) = \frac{Q^{2} e^{-Q^{2} < u_{q}^{2} > /3}}{2n} \times
\]

\[
2 \int_{0}^{\infty} d\omega \frac{e^{-\omega^{2}/K^{2}}}{\sqrt{\pi} \omega^{1/2}} \frac{\omega^{2}V}{2\pi^{2}c^{3}N} (kT/\hbar\omega + 1/2)
\]

(99)

where we have used the condition \( \hbar\omega/kT << 1 \) to replace \( n(\omega) + 1 \) by \( (kT/\hbar\omega + 1/2) \). After evaluating the integral in equation (99), we have:

\[
I_{0}^{\text{inel}}(Q, \omega = 0) = \frac{Q^{2} e^{-Q^{2} < u_{q}^{2} > /3}}{2\pi^{5/2} \rho c\sqrt{\pi} K^{3/2} K^{2/4}} \left[ \frac{kT}{\hbar n} K + K^{2/4} \right]
\]

(100)

where \( \rho = Mn/V \). Then from equations (96), (97) and (100), we get:
\[ R = \frac{Q^2 e^{-q^2 <\Delta^2>/3}}{2\pi^3 \rho c^3} \text{Re}\left\{ \frac{\frac{kTV_\pi}{2\pi} \overline{\rho}(N) + (N)^2/4n}{V\left(\frac{i\Gamma(Q)}{2K}\right)} \right\} \]  

(101)

where \( <\Delta^2> = <u_i^2> - <u_q^2> \). We take \( \rho = 1 \) gm/cm\(^3\), \( c = 1.5 \times 10^5 \) cm/s\(^2\), \( Q \) in A\(^{-1}\), \( \Delta = 0 \), \( \Gamma_r = \frac{\pi^2}{16\pi^2} \) K = 0.66 meV or \( \Gamma_r = 1.0 \times 10^{12} \) rad/s, and for large \( Q \):

\[ \Gamma(Q) = \frac{2DQ^2}{1 + DQ^2\tau_0} + \frac{2}{\tau_0} + \frac{2}{10^{-12}} \text{ s}^{-1} \]  

(102)

Then:

\[ R = 4.6 \times 10^{-5} Q^2 \left( 0.77T + 1 + O(1/T) \right) \]  

(103)

with \( T \) in Kelvin units and \( Q \) in A\(^{-1}\). Hence the approximate relative contribution of the inelastic background to the signal is given by:

\[ C = \frac{4.6 \times 10^{-5} Q^2 \left( 0.77T + 1 \right)}{1 + 4.6 \times 10^{-5} Q^2 \left( 0.77T + 1 \right)} \]  

(104)

and for \( T = 300 \text{K} \) and \( Q^2 = 16 \text{ A}^{-2} \) we get a 15% contribution. For lower temperatures and smaller \( Q \)'s, the contribution is less.
DATA ANALYSIS AND RESULTS

A. Fitting procedure.

The raw data was examined at the time of collection to make sure there were no data points grossly out of line with the other points because of instrumental errors or accidental detection of neutrons from other experiments, for example. For the low resolution or integrated intensity scans, the Bragg peaks from the aluminum sample chamber or from the frozen D$_2$O were deleted from the spectra. Since these peaks shift slightly because of thermal contraction as the temperature is lowered, one must examine points adjacent to the one being deleted to make sure these points are not also affected.

The data was then fit to the appropriate model using a generalized, non-linear least squares algorithm. This algorithm minimizes chi-squared in the parameter space of the function using a set of initial guesses for the parameters. If the guesses are close enough to the best fit parameters, the algorithm converges quickly to the best fit parameters; however, if the guesses are not close enough, the algorithm can give non-physical values of the parameters and a new initial guess must be made. Thus, the fit is considered successful when the parameters are physically reasonable and the reduced chi-squared is less than two. As another check on the validity of the fit, the raw data could be plotted with the fit results and compared, or the residues at each point could be examined for uniformity of size. As a general philosophy, we tried to use as few free parameters in the models as possible.
B. The high resolution experiments.

The high resolution experiments were performed initially to gain information about the diffusive motion of water molecules in a polymeric system. For this reason, most of the data collected were on the deuterated Polyox (or dPolyox) polymer in $\text{H}_2\text{O}$. One solution of hydrogenous polymer in $\text{D}_2\text{O}$ at 10% concentration was done to compare with previous work on a 20% Polyox/$\text{D}_2\text{O}$ solution. We also collected data at 2 Q-values for a 20%dPolyox/$\text{D}_2\text{O}$ solution. This sample was done to get an idea of how much of the scattering could be attributed to the scattering from the deuterons. The results of these two experiments (10% Polyox/$\text{D}_2\text{O}$ and 20% dPolyox/$\text{D}_2\text{O}$) will be presented in the same tables as the results of the dPolyox/$\text{H}_2\text{O}$ experiments.

The water motion was expected to be well described by the jump-diffusion model as discussed earlier (equation (58)) with the possible addition of a contribution to the signal from water tightly "bound" to the surface of the polymer molecules. The separation of the solvent water into "free" and "bound" fractions is called the bound-free model of hydration water and has been frequently utilized as a model for macromolecular-water systems. The bound water is held in place by intermolecular forces such as van der Waals interactions, hydrogen bond forces or electrostatic forces between the water molecules and the macromolecules in solution. In addition, the bound fraction molecules may be expected to physically exchange with the free fraction molecules. The rate of exchange between these two phases then becomes important, and one limit which is commonly assumed in NMR experiments, for example, is the "fast exchange" limit in which the molecules exchange in a time which is very short compared to the
characteristic time of the physical process being observed. However, if the measurement is rapid enough, for example in a high resolution QNS experiment, one would expect to see distinct behavior from each phase of solvent. The water molecules near the surface of macromolecules, then, may be expected to have diffusive properties different from bulk water properties, and this layer of bound water could extend several layers or more into the free fraction. In fact, one might expect the free fraction to be influenced to some degree by long range forces which might occur as a result of the binding of the bound fraction to the macromolecular surface.

Thus, we expect the fixed-Q scans to give a signal:

\[ I_0(Q, \omega) = A + B\omega + R(\omega) \ast \left[ A_0(Q)\delta(\omega) + L(\Gamma_L) \right] \]  \hspace{1cm} (105)

where \( R(\omega) \) is a Gaussian resolution function with FWHM \( \Gamma_{\text{res}} \) (see equation (70)), \( A \) and \( B \) represent the inelastic background, \( A_0(Q)\delta(\omega) \) represents the scattering from the bound fraction of the \( H_2O \), and \( L(\Gamma_L) \) is a Lorentzian with an amplitude of \( L_L \) and a FWHM \( \Gamma_L \) which represents the scattering from the free fraction of the \( H_2O \). The results of fitting the data to equation (105) are shown in Table 4. The values of \( A_0(Q) \) shown in Table 4 are negative in some cases and generally have large uncertainties associated with them even though the values of the reduced chi-squared, \( \chi^2_0 \), are reasonable. Thus, it is not clear that an elastic component in the scattering law is required to interpret the data, and therefore, there is no significant evidence for a bound fraction of the hydration water.
TABLE 4. Results of fitting the high resolution data to equation (105).

<table>
<thead>
<tr>
<th>Sample/ Q(A^{-1})</th>
<th>X2 _ν</th>
<th>1/ν_0</th>
<th>T_{res}</th>
<th>A</th>
<th>-B</th>
<th>A(Q)</th>
<th>L_t</th>
<th>\Gamma_t</th>
<th>1/ν_{_res}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% dPolyox in H_2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0793</td>
</tr>
<tr>
<td>Q = 0.5</td>
<td>1.18</td>
<td>0.0793</td>
<td>97±4</td>
<td>188±28</td>
<td>375±110</td>
<td>1180±170</td>
<td>0.096±0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0.98</td>
<td></td>
<td>109±5</td>
<td>187±28</td>
<td>138±62</td>
<td>826±73</td>
<td>0.139±0.111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.31</td>
<td>&quot;</td>
<td>111±6</td>
<td>201±28</td>
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<td>0.386±0.058</td>
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</table>
We can interpret the data in terms of a pure jump-diffusion model with no bound fraction by fitting the data to:

\[ I_0(Q, \omega) = A + B\omega + R(\omega) \ast \left( L(\Gamma_c) \right) \]  \hspace{1cm} (106)

where the symbols are the same as in equation (105). The results of fitting the data to equation (106) are shown in Table 5.

The values of \( \chi^2 \) in Table 5 are not significantly different from those in Table 4 with the exception of the 38% dPolyox/H\(_2\)O sample where the values are approximately doubled for Q = 0.5 and Q = 0.7. This is accompanied by a reduction of \( \Gamma_L \) for the same sample to values which are a fraction of the resolution FWHM. Thus, one might conclude that for the 38% dPolyox/H\(_2\)O sample, the fit to equation (105) as shown in Table 4 is more plausible than the fit to equation (106) as shown in Table 5. For the other samples, one notes a general reduction in the error associated with each parameter in Table 5 when compared to Table 4 while the values of the parameters are not changed very much. Consequently, we will assume that the fit to the data using equation (106) and the parameters given in Table 5 is adequate when discussing the Q-dependence of the linewidth and the Debye-Waller factors below.

The linewidths are plotted in Figure 3 against Q\(^2\). According to the jump-diffusion model the linewidth is given by:

\[ \Gamma(Q) = \frac{2Q^2D}{1 + Q^2D\tau_0} \]  \hspace{1cm} (59)

The values for the diffusion coefficient, D, and the residence time,
TABLE 5. Results of fitting the high resolution data to equation (106).

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<th>Sample/ ( Q(A^{-1}) )</th>
<th>( x_0^2 )</th>
<th>( \Gamma_{res} ) (meV)</th>
<th>A</th>
<th>-B (THz(^{-1}))</th>
<th>L( t )</th>
<th>( \Gamma_t ) (meV)</th>
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Figure 3. Linewidths versus $Q^2$. dPolyox in H$_2$O at a) 5%, b) 10%, c) 20%, and d) 38% concentration. Polyox in D$_2$O at e) 10% concentration.

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<th>$D \cdot 10^5$ (cm$^2$/sec)</th>
<th>$\tau_0 \cdot 10^{12}$ (sec)</th>
<th>$\langle l^2 \rangle$ (Å$^2$)</th>
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</tbody>
</table>

$\tau_0$, as determined from the data in Table 5 are shown in Table 6. Also shown is the mean-square jump length computed from equation (60). These values may be compared to those determined for pure H$_2$O using a similar experimental technique:$^{132}$ $D \cdot 10^5 = 2.38$, $\tau_0 \cdot 10^{12} = 1.2$, and $\langle l^2 \rangle = 1.7$ Å$^2$. Also the values for a 36% Polyox (WSR-205)/H$_2$O solution (which has the same number of water atoms per monomer as our 38% dPolyox/H$_2$O solution) have been measured using a subtraction technique.$^{134}$ The parameters for solvent water molecules were found by subtracting the spectrum for a Polyox/D$_2$O solution from the spectrum for a Polyox/H$_2$O solution to account for scattering from the polymer protons. The values found were: $D = 0.78 \times 10^{-5}$ cm$^2$/sec and $\tau_0 = 9.8 \times 10^{-12}$ sec. That analysis also found rotational motion with a rotational diffusion coefficient, $D_r = 8.0 \times 10^9$ s$^{-1}$.

The Debye-Waller factors for the Lorentzian in equation (106) are found by fitting the intensity calculated using the parameters of Table 5 to:
I(Q) = I_0 \exp\left(-\langle u^2 \rangle Q^2 / 3 \right) \hspace{1cm} (107)

(Due to the normalization used in the fitting program, the intensity of a Lorentzian component is given by: \( I(Q) = \pi L_t \Gamma_t / 2 \).) The values determined are shown in Table 7, and the intensities are plotted in Figure 4. For the Polyox in D_2O solutions, we have used data for \( Q^2 < 3 \text{ Å}^{-2} \) in the fits reported in Table 7. This is because there seems to be some line-broadening effect around \( Q^2 = 4 \text{ Å}^{-2} \) which may be due to the presence of D_2O. This effect is seen in the integrated intensity data to be presented later. The values of \( \langle u^2 \rangle / 3 \) can be compared to the value determined for H_2O in a similar experiment: \( 133 \text{ Å}^2 \).

<table>
<thead>
<tr>
<th>Sample</th>
<th>( x_0^2 )</th>
<th>( I_0 )</th>
<th>( \langle u^2 \rangle / 3 ) A^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% dPolyox in H_2O</td>
<td>1.63</td>
<td>221\pm12</td>
<td>0.278\pm0.052</td>
</tr>
<tr>
<td>10% dPolyox in H_2O</td>
<td>0.08</td>
<td>119\pm2</td>
<td>0.506\pm0.029</td>
</tr>
<tr>
<td>20% dPolyox in H_2O</td>
<td>0.16</td>
<td>169\pm6</td>
<td>0.574\pm0.038</td>
</tr>
<tr>
<td>38% dPolyox in H_2O</td>
<td>0.55</td>
<td>232\pm9</td>
<td>0.242\pm0.021</td>
</tr>
<tr>
<td>10% Polyox in D_2O</td>
<td>0.27</td>
<td>89\pm4</td>
<td>0.176\pm0.036</td>
</tr>
<tr>
<td>20% Polyox in D_2O</td>
<td>0.04</td>
<td>133\pm4</td>
<td>0.178\pm0.019</td>
</tr>
</tbody>
</table>
Figure 4. Quasi-elastic intensity. dPolyox in H₂O at a) 5%, b) 10%, c) 20%, and d) 38% concentration. Polyox in D₂O at e) 10% concentration.
C. The low resolution experiments.

The empty chamber scans were not strongly dependent on temperature, so we summed all the scans at different temperatures together to get a more statistically reliable set of data for the background signal. After the Bragg peaks were eliminated, we fit the background data to an empirical formula which seemed to describe the data adequately:

\[ I_{\text{bkg}}(Q) = A_b + B_b Q^\alpha + C_b Q^\beta + D_b L_1(Q_1, \Gamma_1) + E_b L_2(Q_2, \Gamma_2) \]  

(108)

The least squares fit gives \( \chi^2_0 = 3.81 \) with \( A_b = 5.74 \pm 2.3 \), \( B_b = -4.065 \pm 3.1 \), \( \alpha = 4.666 \pm 0.67 \), \( C_b = 3.801 \pm 3.0 \), \( \beta = 4.718 \pm 0.63 \), \( D_b = 59.9 \pm 13 \), \( Q_1 = 0.236 \pm 0.1 \), \( \Gamma_1 = 0.571 \pm 0.058 \), \( E_b = 29.3 \pm 8 \), \( Q_2 = 2.91 \pm 0.040 \), and \( \Gamma_2 = 0.872 \pm 0.096 \). \( L(Q_0, \Gamma) \) is a Lorentzian with unit amplitude centered at \( Q_0 \) and a FWHM \( \Gamma \). This empirical function is expected to be valid only for \( 0.3 \leq Q \leq 4.1 \) and \( T \leq 300K \), and it is normalized to the same incident flux as the data to be presented (Monitor = 100 with Monitor Count Unit = 5000). Although the value of \( \chi^2_0 \) is not particularly impressive for this fit and there seems to be a large uncertainty in some of the parameters in equation (108), this background correction was chosen rather than a point by point subtraction because of the low count rate in the background scans. This background function has been subtracted from all of the data to be presented. The background data and the fit to the data are shown in Figure 5.
Figure 5. Summed background scans and least-squares fit.

Coherent scattering from crystalline samples at certain $Q$-values produces Bragg peaks. The condition for Bragg scattering is that:

$$\mathbf{Q} = \mathbf{\hat{r}}$$

(109)

where $\mathbf{\hat{r}}$ is any vector of the reciprocal lattice. For a powder sample, the vector notation in equation (109) may be dropped and we have a Bragg peak whenever $Q = \tau$. The reciprocal lattice of a Bravais space lattice is a Bravais lattice; hence, all of the points in the reciprocal lattice are given by:

$$\mathbf{\hat{r}} = n_1 \mathbf{\hat{a}}_1 + n_2 \mathbf{\hat{a}}_2 + n_3 \mathbf{\hat{a}}_3, \quad n_i = 0, 1, 2, ...$$

(110)

where the $\mathbf{\hat{a}}_i$ are primitive vectors for the reciprocal lattice. Then the magnitude of $\mathbf{\hat{r}}$ is:
\[
\tau = \left( \frac{3}{2} n_1 n_2 \hat{a}_i \cdot \hat{a}_j \right)^{1/2}. \tag{111}
\]

Any integer multiple of \( \tau \) is also the magnitude of a reciprocal lattice vector. By evaluating equation (111) for various materials in the sample, we can identify the origin of any Bragg peaks we observe.

For aluminum, the space unit cell is face-centered cubic (FCC) and the reciprocal unit cell is body-centered cubic (BCC). A choice for the primitive vectors of the reciprocal lattice is:

\[
\hat{a}_1 = \frac{4\pi}{a} \hat{x}, \quad \hat{a}_2 = \frac{4\pi}{a} \hat{y}, \quad \hat{a}_3 = \frac{2\pi}{a} (\hat{x} + \hat{y} + \hat{z}) \tag{112}
\]

Then the values of \( \tau \) are:

\[
\tau = \frac{\hbar \pi}{a} \left[ (n_1 + n_3/2)^2 + (n_2 + n_3/2)^2 + n_3^2/4 \right]^{1/2} \tag{113}
\]

Since we are interested in \( Q < 4 \text{ \AA}^{-1} \), we can substitute values for the \( n_i \) to find values of \( Q \) where Bragg peaks are possible. The results are shown in Table 8.

For \( D_2O \) ice, the space unit cell is simple hexagonal as is the reciprocal lattice.\(^{137}\) A choice for the primitive vectors of the reciprocal lattice is:

\[
\hat{a}_1 = \frac{4\pi}{\sqrt{3}a} \hat{x}, \quad \hat{a}_2 = \frac{2\pi}{\sqrt{3}a} \hat{x} + \frac{2\pi}{a} \hat{y}, \quad \hat{a}_3 = \frac{2\pi}{c} \hat{z} \tag{114}
\]

Then the values of \( \tau \) are:
TABLE 8. Possible Bragg peak positions with $\tau \leq 4$ A$^{-1}$.

<table>
<thead>
<tr>
<th>Material</th>
<th>Lattice Constants (Å)</th>
<th>$(n_1, n_2, n_3)$</th>
<th>$\tau$ (Å$^{-1}$)</th>
<th>Observed in D$_2$O sample?</th>
<th>$Q_{obs}$ (Å$^{-1}$)</th>
<th>$T_{obs}$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum$^{138}$</td>
<td>$a = 4.05$ (300K)</td>
<td>$(0,0,1)$</td>
<td>2.6871</td>
<td>Yes</td>
<td>$2.6857 \pm 0.0002$</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,0,2)$</td>
<td>3.1028</td>
<td>Yes</td>
<td>$3.1003 \pm 0.0002$</td>
<td>300</td>
</tr>
<tr>
<td>D$_2$O Ice$^{137}$</td>
<td>$a = 4.48$ (98K)</td>
<td>$(0,0,1)$</td>
<td>0.8595</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,1,0)$</td>
<td>1.6195</td>
<td>Yes</td>
<td>$1.6052 \pm 0.0002$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,0,1)$</td>
<td>1.7191</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,1,1)$</td>
<td>1.8334</td>
<td>Yes</td>
<td>$1.8183 \pm 0.0001$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,1,2)$</td>
<td>2.3617</td>
<td>Yes</td>
<td>$2.3426 \pm 0.0003$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,0,3)$</td>
<td>2.5786</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(1,1,0)$</td>
<td>2.8050</td>
<td>Yes</td>
<td>$2.7799 \pm 0.0007$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(1,1,1)$</td>
<td>2.9337</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,1,3)$</td>
<td>3.0450</td>
<td>Yes</td>
<td>$3.0195 \pm 0.0002$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,2,0)$</td>
<td>3.2389</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(1,1,2)$</td>
<td>3.2899</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,2,1)$</td>
<td>3.3510</td>
<td>Yes</td>
<td>$3.3209 \pm 0.0005$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,0,4)$</td>
<td>3.4381</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,2,2)$</td>
<td>3.6669</td>
<td>Yes</td>
<td>$3.6340 \pm 0.0007$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(0,1,4)$</td>
<td>3.8004</td>
<td>Yes</td>
<td>$3.77$</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(1,1,3)$</td>
<td>3.8101</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$$\tau = 2\pi \left[ \frac{4}{3} \frac{a^2}{a^2} (n_1 + n_2/2)^2 + n_2^2/a^2 + n_3^2/c^2 \right]^{1/2} \quad (115)$$

The possible Bragg peaks with $Q < 4$ Å$^{-1}$ are shown in Table 8.

Table 8 indicates that all of the possible Bragg peaks are not observed in the D$_2$O sample. This may be explained by the fact that the intensities will be influenced by the structure factor for the Bragg peaks. Another possibility is that some of the peaks are so narrow that they were never within the resolution window. Support for the former possibility is given in Figure 6. Here we have plotted high Q-resolution (Q step size = .01 Å$^{-1}$) scans for the D$_2$O sample at 250K
Figure 6. High Q-resolution scans. a) D\textsubscript{2}O at 250K. b) Raw trypsin in D\textsubscript{2}O at 250K.

(Figure 6(a)) and for the raw trypsin in D\textsubscript{2}O at 250K (Figure 6(b)). There are some differences in the amplitudes of the D\textsubscript{2}O Bragg peaks, and the D\textsubscript{2}O Bragg peak near Q = 1.72 Å\textsuperscript{-1} is apparent in Figure 6(b) but not in Figure 6(a). Thus, by incorporating trypsin into the frozen D\textsubscript{2}O, the structure factor is changed because the atoms in the unit cell must move slightly to accommodate the trypsin. This effect is also seen in the Polyox/D\textsubscript{2}O sample.

The contribution to the signal from scattering from the D\textsubscript{2}O at other than the Bragg peaks was significant in the polymer/D\textsubscript{2}O solutions because of the relative abundance of deuterons. The spectra from D\textsubscript{2}O were measured in the same manner as the other spectra, and it was found that the D\textsubscript{2}O signal (minus the Bragg peaks) could be fit with a function:

\[
I_D(Q) = A_d + B_d \cdot L(T,Q_0) + C_d \cdot V(18Q)
\]  

(116)
where

\[ b_q = \frac{b_a^2 q^2}{1 + b_b^2 q^2} \]  \hspace{1cm} (117)

\( V(z) \) is the Voigt function (equation (75)), and \( L(\Gamma, Q_0) \) is a unit amplitude Lorentzian with a FWHM \( \Gamma \) centered at \( Q_0 \). Equation (116) was chosen to represent empirically the \( D_2O \) spectra for two reasons. The constant term and the Voigt function term were chosen as a pair to represent the incoherent scattering from the sample at all temperatures. Below 225K this scattering gives a relatively constant intensity because the quasi-elastic linewidth is small, while above 225K, there is a \( Q \)-dependence to the intensity because of the \( Q \)-dependence of the linewidth which is assumed to have the form shown in equation (117). Thus, at a given temperature, either \( A_d \) or \( C_d \) is chosen to be zero. The Lorentzian term was included to account for the non-Bragg coherent scattering. This scattering is generally oscillatory in \( Q \) with an oscillation amplitude which decreases with increasing \( Q \). We have accounted for the first maximum in this oscillating function by including the Lorentzian term in equation (116). The results of fitting the \( D_2O \) spectra to equation (116) are shown in Table 9. These parameters were used as fixed parameters to subtract the \( D_2O \) contribution to the signal in all of the polymer solutions studied. The data and fit at two temperatures is shown in Figure 7. Unfortunately, we did not collect the \( D_2O \) data below 150K, so when the fits to the polymer/\( D_2O \) solutions below 150K were run, it became necessary to include some of the parameters in equation (116) as
TABLE 9. Results of fitting the D$_2$O spectra to equation (116).

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>$\chi^2_\nu$</th>
<th>$A_d$</th>
<th>$B_d$</th>
<th>$Q_0$ (Å$^{-1}$)</th>
<th>$\Gamma$ (Å$^{-1}$)</th>
<th>$C_d$</th>
<th>$\beta_a$</th>
<th>$\beta_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0.68</td>
<td>0</td>
<td>94±5</td>
<td>1.92±.02</td>
<td>0.75±.04</td>
<td>60±14</td>
<td>1.31±.42</td>
<td>0.67±.19</td>
</tr>
<tr>
<td>285</td>
<td>0.94</td>
<td>0</td>
<td>116±7</td>
<td>1.90±.02</td>
<td>0.79±.04</td>
<td>76±32</td>
<td>1.89±.89</td>
<td>1.02±.32</td>
</tr>
<tr>
<td>275</td>
<td>0.66</td>
<td>0</td>
<td>150±6</td>
<td>1.91±.01</td>
<td>0.70±.03</td>
<td>61±18</td>
<td>1.43±.69</td>
<td>1.08±.37</td>
</tr>
<tr>
<td>250</td>
<td>1.46</td>
<td>0</td>
<td>84±6</td>
<td>3.10±.05</td>
<td>1.48±.23</td>
<td>120±25</td>
<td>1.26±.47</td>
<td>0.88±.56</td>
</tr>
<tr>
<td>225</td>
<td>0.82</td>
<td>49±4</td>
<td>39±7</td>
<td>3.13±.10</td>
<td>1.52±.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>0.76</td>
<td>43±8</td>
<td>43±7</td>
<td>3.19±.14</td>
<td>2.51±.47</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>175</td>
<td>0.91</td>
<td>56±3</td>
<td>62±7</td>
<td>3.31±.06</td>
<td>0.94±.10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>0.67</td>
<td>48±5</td>
<td>51±5</td>
<td>3.48±.12</td>
<td>2.10±.25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 7. Example D$_2$O spectra and least-squares fit.

free parameters to account for the D$_2$O contribution to the signal. Typically, reliable fits were obtained at these low temperatures only with the inclusion of the Lorentzian in equation (116); if any more parameters were included, the fit often would not converge.
We discuss the fit results for the polymer powders next since they do not have a D$_2$O contribution to the scattering. Also, we can evaluate the importance of the inelastic background contribution to the signal. The quasi-elastic linewidth of a solid is much narrower than a liquid; the scattering law for incoherent elastic scattering is given by equation (33) or (34). That the quasi-elastic linewidth of a solid is narrower than that of a liquid can also be shown by considering the scattering laws which we have already written down for translational diffusion in the limit of large residence time and reduced diffusion constant (see equations (52) or (59) for example). For the powder samples, then, the quasi-elastic peak will look like a $\delta(\omega)$ function compared to our broad resolution function. Thus, we expect the quasi-elastic peak to give a contribution to the signal like the first term in equation (82). The inelastic background will give a contribution with a Q dependence like equation (100). Thus, we attempted to fit the polymer powder, low resolution data to:

$$I_{\text{Powder}}(Q) = A e^{-<u_{\text{qe}}^2>Q^2/3} + B_{\text{in}} e^{-<u_{\text{in}}^2>Q^2/3}$$

(118)

The results of this attempt for the Polyox (8000) powder data are shown in Table 10. The fits to the data shown in Table 10 are not very satisfactory because there are some large uncertainties associated with some parameters, and there are wild fluctuations in some parameters as the temperature is decreased. However, one feature is apparent: the amplitude of the inelastic background contribution is small in relation to the quasi-elastic contribution. For this reason, we fit the same
TABLE 10. Results of fitting the Polyox (8000) powder spectra to equation (118).

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>$x_0^2$</th>
<th>$A_0$</th>
<th>$&lt;u_{q_0}&gt;/3$ (Å²)</th>
<th>$B_{in}$</th>
<th>$&lt;u_{in}&gt;/3$ (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>1.21</td>
<td>865±15</td>
<td>0.178±0.068</td>
<td>59±47</td>
<td>0.093±0.036</td>
</tr>
<tr>
<td>285</td>
<td>1.51</td>
<td>842±15</td>
<td>0.079±0.047</td>
<td>2±31</td>
<td>0.01±0.5</td>
</tr>
<tr>
<td>275</td>
<td>1.53</td>
<td>878±19</td>
<td>0.260±0.066</td>
<td>137±41</td>
<td>0.131±0.017</td>
</tr>
<tr>
<td>250</td>
<td>1.48</td>
<td>891±18</td>
<td>0.245±0.063</td>
<td>136±40</td>
<td>0.119±0.016</td>
</tr>
<tr>
<td>225</td>
<td>0.97</td>
<td>881±14</td>
<td>0.17±0.11</td>
<td>101±82</td>
<td>0.101±0.031</td>
</tr>
<tr>
<td>200</td>
<td>1.19</td>
<td>917±16</td>
<td>0.202±0.058</td>
<td>123±40</td>
<td>0.096±0.016</td>
</tr>
<tr>
<td>175</td>
<td>1.05</td>
<td>950±15</td>
<td>0.183±0.066</td>
<td>128±50</td>
<td>0.093±0.018</td>
</tr>
<tr>
<td>150</td>
<td>1.58</td>
<td>973±14</td>
<td>0.04±0.18</td>
<td>8±300</td>
<td>0.04±0.7</td>
</tr>
<tr>
<td>125</td>
<td>1.91</td>
<td>1017±22</td>
<td>0.217±0.066</td>
<td>181±48</td>
<td>0.096±0.014</td>
</tr>
<tr>
<td>100</td>
<td>1.71</td>
<td>1030±22</td>
<td>0.247±0.052</td>
<td>217±34</td>
<td>0.095±0.010</td>
</tr>
</tbody>
</table>

The results of this fit were more satisfactory, and we fit all of the powder data sets to:

$$I_{\text{Powder}}(Q) = A_0 e^{-<u^2>Q^2/3}, \quad (118a)$$

where $<u_{q_0}^2>$ has been replaced by $<u^2>$, since it may no longer represent the true mean-square thermal motion, due to the influence of the omitted inelastic background. The results are shown in Table 11. Although the values of $x_0^2$ are slightly larger for the Polyox (8000) fit in Table 11 compared to the values in Table 10, the parameters change with decreasing temperature in a reasonable manner and the associated errors are small. Also, the values of $<u^2>/3$ are smaller than $<u^2>/3$ for $H_2O$, which is what one expects for solids.
TABLE 11. Results of fitting the powder spectra to equation (118a).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temp (K)</th>
<th>$x_0^2$</th>
<th>$A_0$</th>
<th>$&lt;u^2&gt;/3$ (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyox (8000)</td>
<td>300</td>
<td>1.75</td>
<td>827±11</td>
<td>0.077±0.002</td>
</tr>
<tr>
<td></td>
<td>285</td>
<td>1.46</td>
<td>836±10</td>
<td>0.072±0.002</td>
</tr>
<tr>
<td></td>
<td>275</td>
<td>1.59</td>
<td>851±10</td>
<td>0.068±0.002</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.57</td>
<td>865±10</td>
<td>0.058±0.002</td>
</tr>
<tr>
<td></td>
<td>225</td>
<td>0.96</td>
<td>861±8</td>
<td>0.051±0.001</td>
</tr>
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<td>107±3</td>
<td>0.061±0.005</td>
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<tr>
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<td>0.077±0.009</td>
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<td>60±2</td>
<td>0.063±0.007</td>
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<td>0.038±0.007</td>
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<td>0.028±0.005</td>
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<td>59±2</td>
<td>0.028±0.006</td>
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<td>100</td>
<td>0.97</td>
<td>58±3</td>
<td>0.013±0.006</td>
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<td>75</td>
<td>0.93</td>
<td>58±3</td>
<td>0.019±0.006</td>
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</table>
The temperature dependence of $\langle u^2 \rangle / 3$ for the powder samples is shown in Figure 8 along with a linear fit to the data:

$$\langle u^2 \rangle / 3 = A_u T + B_u$$  \hspace{1cm} (119)

The coefficients of this fit are shown in Table 12. The parameters for the two trypsin samples in Table 12 seem to have quite a bit of overlap.

Figure 8. $\langle u^2 \rangle / 3$ for the powder samples. a) Polyox (8000), b) Raw trypsin, c) Treated trypsin.
TABLE 12. Results of fitting the polymer powder data from Table 11 to equation (119).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$x_0^2$</th>
<th>$A_U \cdot 10^4$ (Å$^2$/K)</th>
<th>$-B_U \cdot 10^2$ (Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyox (8000)</td>
<td>1.36</td>
<td>2.98±.10</td>
<td>1.51±.20</td>
</tr>
<tr>
<td>Raw Trypsin</td>
<td>0.34</td>
<td>2.19±.09</td>
<td>0.80±.19</td>
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<tr>
<td>Treated Trypsin</td>
<td>1.48</td>
<td>2.50±.34</td>
<td>0.61±.65</td>
</tr>
</tbody>
</table>

while the Polyox parameters show little overlap with the trypsin samples. This is further evidence that the two trypsin samples are very similar. A problem with the data in Table 12 is that the $B_U$'s are negative. This is probably due to the influence of the inelastic background and will be addressed later.

It was very difficult to obtain consistent, reasonable fit results for the polymer/D$_2$O samples, although we eventually were able to obtain values for $<U^2>$. An inelastic background term was not included in our fits because we have previously found it to make a small contribution to the signal, and the data does not have high enough statistical reliability to determine those parameters. It was necessary to include parametrically the contribution from D$_2$O scattering, as well as some contribution due to the non-ideal resolution function. The fitting function we used to fit the polymer/D$_2$O data is:

$$I_{sol}(Q) = D_0 \cdot I_d(Q) + K_0 \cdot e^{-<U^2>Q^2/3} \cdot V(i8_g)$$  (120)
where $I_d(Q)$ is given by equation (116) and the data in Table 9, and where $\beta_s$ is given by:

$$
\beta_s = \frac{\beta_1^2Q^2}{1 + \beta_2^2Q^2}
$$

(121)

$D_0$ represents the fraction of the signal which comes from the $D_2O$ in the sample, and this was taken as one of the free parameters in the fit. Since the amount of $D_2O$ was constant throughout the experiment, we would expect $D_0$ to be relatively constant as the temperature is lowered. This is what was found, but the error associated with this parameter is not small in all cases. The parameters $\beta_1$ and $\beta_2$ represent the effect of the convolution of the quasi-elastic linewidth with the resolution function. As the temperature is lowered, the linewidth ($\Gamma$) becomes narrower so that at some temperature the approximation $\Gamma_{\text{resolution}} >> \Gamma$ becomes valid (see equation (82)), and then $V(\beta_s) \rightarrow 1$. Thus, as we tried to fit the data at lower and lower temperatures, we would eventually choose $\beta_1 = 0$ and $\beta_2 = 0$ when the fit would not converge properly. The parameters resulting from this fitting procedure are shown in Table 13.

The temperature dependence of $\langle U^2 \rangle$ is similar to the form for the powder samples, except for the trypsin/$D_2O$ samples which show an inflection at the freezing point of water. This data is shown in Figure 9. Perhaps there is also a freezing point inflection in the Polyox data, but the error bars are so large that this feature would be hidden. The fit to equation (119) gives the parameter values shown in Table 14. The errors associated with the parameters in Table 14 are
TABLE 13. Results of fitting the polymer/D$_2$O spectra to equation (120).

<table>
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<tr>
<th>Sample</th>
<th>Temp (K)</th>
<th>$\chi^2$</th>
<th>$D_0$</th>
<th>$K_0$</th>
<th>$&lt;u^2&gt;/3$</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
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</thead>
<tbody>
<tr>
<td>Polyox (WSR-205)/D$_2$O</td>
<td>300</td>
<td>1.27</td>
<td>1.79±.22</td>
<td>560±31</td>
<td>0.057±.040</td>
<td>0.565±.069</td>
<td>0.32±.19</td>
</tr>
<tr>
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<td>300</td>
<td>1.39</td>
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<td>543±33</td>
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<td>0.614±.066</td>
<td>0.22±.34</td>
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</tr>
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<td>275</td>
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<td>1.44±.16</td>
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<td>0.61±.28</td>
<td>0.82±.49</td>
</tr>
<tr>
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Continued
### TABLE 13. (Continued).

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</tbody>
</table>

$^a$At these temperatures, the D$_2$O contribution was given by a Lorentzian only, with amplitude, width, and position as free parameters.

$^b$These fits were for data with $Q > 0.5$.

### TABLE 14. Results of fitting the polymer/D$_2$O data from Table 13 to equation (119).

<table>
<thead>
<tr>
<th>Sample</th>
<th></th>
<th>$x_0^2$</th>
<th>$A_{u} \cdot 10^4$ (Å²/K)</th>
<th>$-B_{u} \cdot 10^2$ (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyox (WSR=205)/D$_2$O</td>
<td></td>
<td>1.63</td>
<td>4.06±50</td>
<td>2.28±81</td>
</tr>
<tr>
<td>Polyox (8000)/D$_2$O</td>
<td></td>
<td>4.59</td>
<td>5.0±1.1</td>
<td>4.8±2.1</td>
</tr>
<tr>
<td>Raw Trypsin/D$_2$O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T≥275K</td>
<td></td>
<td>0.14</td>
<td>5.65±66</td>
<td>8.8±1.9</td>
</tr>
<tr>
<td>T&lt;275K</td>
<td></td>
<td>0.58</td>
<td>2.20±25</td>
<td>1.93±41</td>
</tr>
<tr>
<td>Treated Trypsin/D$_2$O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T≥275K</td>
<td></td>
<td>1.11</td>
<td>4.7±1.8</td>
<td>6.8±5.1</td>
</tr>
<tr>
<td>T&lt;275K</td>
<td></td>
<td>0.10</td>
<td>1.88±0.08</td>
<td>1.36±1.0</td>
</tr>
</tbody>
</table>
Figure 9. $<\mathbf{u}^2>/3$ for the solutions. a) Polyox (WSR-205)/D$_2$O, b) Polyox (8000)/D$_2$O, c) Raw Trypsin/D$_2$O, d) Treated Trypsin/D$_2$O.

large in some cases, but a few points may be made. The values of $<\mathbf{u}^2>$ are clearly different above and below freezing for the trypsin samples, and the frozen sample values are similar to the powder values in Table 12. Also, there is not much difference in the $A_u$'s for all of the samples above 275K. Again, the $B_u$'s are negative, as in Table 12.
CONCLUSIONS

A. Discussion of the high energy-resolution experiments.

The primary results of the high energy-resolution experiments, which were performed only on Polyox solutions, are contained in Tables 5, 6, and 7, and in Figures 3 and 4. We have concluded that there is no evidence for a narrow line contribution from a bound fraction of the hydration water in the dPolyox/H$_2$O solutions with the possible exception of the 38% dPolyox/H$_2$O solution. Therefore, we have interpreted all of the data within the simple jump-diffusion model for the motion of the scattering protons which gives us an "effective" diffusion coefficient and residence time with no bound fraction of the hydration water.

This interpretation of the data, however, does not rule out the existence of a bound fraction of water. One of the assumptions of the bound-free model is that the macromolecule to which the water is bound is relatively immobile compared to bulk water molecules. It is the nearly static structure of the macromolecules which effectively reduces the diffusive properties of the water molecules bound to its surface. The Polyox in our solutions is, however, quite mobile at 10% and 20% concentration. The diffusion coefficients and residence times shown in Table 6 for Polyox (WSR-205) in D$_2$O differ from the values for H$_2$O only by a factor of about 2. The work of Lang, et al.,$^{78}$ using carbon-13 NMR and ESR on spin-labelled Polyox at about 10% concentration shows that the segmental motion of Polyox seems to be well described by an isotropic rotation model which indicates that the polymer is highly flexible. Since the polymer is apparently very mobile, any protons bound to the surface would also appear quite mobile and thus would not
necessarily be distinguishable as a narrow line in our experiment. If the polymer were immobilized somehow, the distinction between bound and free water would become more apparent. One way to immobilize the polymer would be to raise the concentration of the polymer in the solvent until the polymer forms a network which is entangled or cross-linked by hydrogen bonds. This may be what happens in the 38% dPolyox/D$_2$O solution since we have some indication of bound water in that sample. If this picture is valid, the motion we see in the solvent molecules is an average of the bound and free fraction parameters.

The diffusive motion of the H$_2$O protons in the 5% dPolyox/H$_2$O solution is very similar to that of bulk water as indicated by the values of D and $\tau_0$ in Table 6. For the 10% and 20% dPolyox/H$_2$O solutions the fit to the $f(Q)$ data seems to indicate that the diffusion coefficient and the residence time are both increased over the pure water value. The most likely explanation for this is that the linewidth at small Q-values has been overestimated (giving a larger effective diffusion coefficient) because of the simplified form of our fitting function.

We have fit the quasi-elastic peak, which may be a sum of several Lorentzian components, with a single Lorentzian term which gives us an "effective" linewidth which must be larger than the linewidth due to translational motion. For example, if isotropic rotational motion characterized by a rotational diffusion coefficient, $D_R$, is present in addition to translational jump diffusion, the scattering law from equation (18) is:
\[ S^d-R(Q, \omega) = n S^d_1(Q, \omega) \ast S^R_1(Q, \omega) \]  
(122)

where \( S^d_1(Q, \omega) \) is given by equation (58) and \( S^R_1(Q, \omega) \) is given by equation (61). The scattering law is:

\[
S^d-R(Q, \omega) = \frac{e^{-Q^2\langle u^2 \rangle / 3}}{\pi n} \left\{ \frac{A_0 \Gamma/2}{(\Gamma/2)^2 + \omega^2} + \sum_{\ell=1} A_\ell \frac{\ell(\ell+1)D_R + \Gamma/2}{(\ell(\ell+1)D_R + \Gamma/2)^2 + \omega^2} \right\} \]  
(123)

where \( A_\ell \) is given in equation (62) and \( \Gamma \) depends on \( Q \) according to equation (59). The first two terms of equation (123) have been used in a computer simulation by Heidorn to study the effect of a simplified fitting function in frog muscle spectra.\textsuperscript{139} Data was generated using a scattering law similar to equation (123) which was convoluted with a Gaussian resolution function. Values of physical parameters such as the translational and rotational diffusion coefficients were chosen consistent with fit results on the frog muscle samples. After the data was generated, it was fit with a fitting function which did not include the rotational scattering law, and then the linewidths were fit to the jump-diffusion model (equation (59)). This simulation showed that the effective diffusion coefficients calculated from the simplified fitting function were consistently larger than the actual value chosen at the start of the simulation by a factor of more than 2. This is because of the importance of low Q-value data in estimating \( D_t \) and the fact that the low Q-value linewidths are always overestimated by a larger
percentage than high Q-value linewidths when the fitting function is 
simplified in this manner. However, it was also shown that the 
residence time, $\tau_0$, which depends more on the linewidth at large 
Q-values, was more consistently estimated than $D_L$ by the simplified 
fitting function. Thus, we may conclude that the elevated values of 
the diffusion parameters for the 10% and 20% dPolyox/D$_2$O solutions are 
probably a result of a simplified fitting function. Other studies 
support this conclusion; Heatly and Walton$^{79}$ point out that some NMR 
studies of solvents show essentially no effect of solute on solvent 
motion up to concentrations of 20 to 30%. We would need data with 
better statistics to be able to do a proper fit to equation (123) to 
separate the translational and rotational contributions to the motion.
The time-of-flight IN$^6$ spectrometer at the Institut Laue-Langevin (ILL) 
in Grenoble, France would be the spectrometer of choice for this 
experiment.$^{120}$

Another possibility, though less likely than the above 
explanation, is that the line broadening at small Q-values for these 
two concentrations is a real effect attributable to some kind of 
"structure breaking" effect that the polymer has on the water. Pure 
water is very structured because of the strong hydrogen bonding which 
causes clusters or clumps of molecules to form with near tetrahedral 
symmetry on a time scale which is long compared to the intramolecular 
vibrational time.$^{140}$ If this structure is perturbed so that the water 
protons have a larger translational mobility, the average jump distance 
might increase. This increase in the diffusion coefficient was seen in 
a computer simulation of water near a protein surface,$^{21}$ and was
attributed to the type of structure breaking effect we are considering here.

In the case of the 38% dPolyox/H₂O solution, the effective diffusion coefficient is reduced by about a factor of 2.5 from the bulk water value while the residence time is increased by a factor of about 2.1. The net result is that there is no significant change in the mean-square jump length compared to pure water. Therefore, at this concentration we may assert that there is a true reduction of the diffusive freedom of the H₂O protons. Two different mechanisms for this reduction of the effective diffusion coefficient are conceivable and, in fact, both may occur in our sample. The first mechanism was mentioned in the discussion about the possible existence of a bound fraction in the 38% solution: we hypothesized that there is a bound fraction of water which is not apparent at lower concentrations because of the mobility of the polymer, but at higher polymer concentrations, the polymer becomes less mobile because of cross linking and entanglements. Thus, the diffusive motion of the bound water is reduced, and this reduces our effective diffusion parameters. The second explanation is that there is a "stiffening" of the bulk water structure alluded to earlier. A plausible way for this to happen is that the polymer forms a network because of entanglements between polymer chains. Then groups of water molecules could become clustered in pockets or spaces formed by the polymer network. The interaction of the water molecules with the polymer surface lining the pockets could have an ordering effect on the water. Liu and Anderson\textsuperscript{77} point out that several experimental techniques imply that water molecules, more than other solvents, promote an ordered structure in Polyox. We are
merely suggesting the water is also structured by the polymer/solvent interaction.

In summary, the diffusive properties of water in these Polyox/water solutions is slightly affected at low (5%) and intermediate (10-20%) polymer concentrations. It is difficult to estimate the diffusion coefficient with much certainty because of the inherent difficulties of QNS at low Q-values. However, the linewidth at large Q-values is significantly reduced from the pure water values and indicates an increased residence time, $\tau_0$, for these samples. At high polymer concentration (38%), the water clearly has a reduced diffusion coefficient and an increased residence time compared to bulk water and this may be attributed to the nature of the interaction of the water molecules with the polymer.

B. Discussion of the low energy-resolution experiments.

The primary results of the low energy-resolution experiments are contained in Tables 11, 12, 13, and 14, and in Figures 8 and 9. We have been able to determine an effective mean-square amplitude, $\langle \Delta U^2 \rangle / 3$, for each of our samples as a function of temperature. The temperature dependence of $\langle \Delta U^2 \rangle / 3$ is of the form $A_u T + B_u$ (equation (119)) for all of the samples with $A_u > 0$ and $B_u < 0$; the sign of $B_u$ is not consistent with the interpretation of $\langle \Delta U^2 \rangle$ as the mean-square amplitude of the thermal vibration. The most probable explanation for the negative values of $B_u$ is our treatment of the inelastic background.
1. The inelastic background.

The primary source for our negative intercept for the values of $<u^2>/3$ is the inelastic background. Earlier, we estimated the inelastic contribution to the scattering intensity by examining the one phonon spectrum of a Debye solid near $\omega = 0$. We estimated that this contribution to the scattering intensity was small. In fact, in the data analysis for the powder samples, it was apparent that the inelastic background was small. We can evaluate the temperature dependence of this model for the inelastic background to see if this model gives the proper sign and magnitude of correction necessary to make our data appear more reasonable. The total scattering intensity was written in equation (94) as:

\[
I_{\gamma}(Q, \omega = 0) + I_{\gamma}^{\text{inel}}(Q, \omega = 0) = I_{\gamma}(Q, \omega = 0) (1 + R)
\]  \hspace{1cm} (124)

where $R$ is defined in equation (96). From equation (103) we have:

\[
I_{\gamma}(Q, \omega = 0) (1 + R)
\]

\[
= I_{\gamma}(Q, \omega = 0) \left(1 + Q^2(3.5 \times 10^{-5}T + 4.6 \times 10^{-5})\right). \hspace{1cm} (125)
\]

Remembering the expansion $e^x = 1 + x + \cdots$, we write equation (125) as:

\[
= I_{\gamma}(Q, \omega = 0) e^{Q^2(3.5 \times 10^{-5}T + 4.6 \times 10^{-5})}. \hspace{1cm} (126)
\]

Thus, when we ignore the inelastic contribution to the scattering, we have an effective mean-square amplitude:

\[
<u^2>/3 = <u^2>/3 - 3.5 \times 10^{-5}T - 4.6 \times 10^{-5} \hspace{1cm} (127)
\]
with $<u^2>$ in $\text{Å}^2$ and $T$ in Kelvin. Thus at 300K, we must add an estimated correction of 0.011 $\text{Å}^2$ to our calculated values of $<u^2>/3$. This is a sizeable correction at 300K (about 11% for the Polyox in D$_2$O sample), but the constant term in equation (127) is too small to adequately explain the negative values of $B_u$. Thus, this model of the inelastic background does not completely explain our results, but the sign of the correction is in the right direction. One must remember that we used a model for the inelastic background which may be far from reasonable. That is, the concept of phonons in these non-crystalline samples may be a very rough approximation to the types of motions possible in these molecules. The modeling of the intramolecular motion by harmonic restoring forces has been shown in computer simulations to yield results that agree poorly with inelastic neutron spectra.\textsuperscript{141} Thus, it is possible that the anharmonic terms are very important.

We have another way of estimating the effect of the inelastic background on our values of $<u^2>/3$: we can examine the inelastic background which was measured in the high energy-resolution experiments and use those measured parameters to calculate the inelastic contribution to the total scattering for an arbitrary resolution function FWHM, $\Gamma_{\text{res}}$. To do this we need to write an expression for the scattering intensity for an arbitrary $\Gamma_{\text{res}}$. In the high energy-resolution experiments we have a scattering law:

$$s_{\text{tot}}(Q,\omega) = s_{\text{inel}}(Q,\omega) + s_{\text{qe}}(Q,\omega)$$  (128)

which must be convoluted with the resolution function, $R(\omega)$, which is shown in equation (70), so that we have a function proportional to the
signal detected at a given $Q$ and $\omega$. We have found that the inelastic background was adequately accounted for by a linear function of $\omega$. That is:

$$I_0^{\text{inel}}(Q, \omega) = R(\omega) \ast S^{\text{inel}}(Q, \omega)$$

$$= A(Q) + B(Q)\omega,$$

when the resolution function had a FWHM $\Gamma_{\text{res}} = \Gamma_{\text{hi}}$. The fit values of $A(Q)$ and $B(Q)$ are shown in Table 5 for the samples we examined. It is easily shown that for this form of the inelastic background $A(Q)$ and $B(Q)$ do not depend on the width of the resolution function, $\Gamma_{\text{res}}$, as long as the inelastic background varies slowly with $\omega$. For the quasi-elastic contribution to the intensity we take:

$$I_0^g(Q, \omega) = R(\omega) \ast S^g(Q, \omega)$$

$$= \frac{L_t}{\sqrt{\pi} K} \left(\frac{\Gamma_t}{2}\right)^2 \int_{-\infty}^{\infty} \frac{dx}{x^2 + (\Gamma_t/2)^2} e^{-(\omega-x)^2/K^2}$$

(130)

where, from equation (71),

$$K = \Gamma_{\text{res}}/2\sqrt{\ln 2},$$

and we have assumed that the quasi-elastic scattering law is a Lorentzian with FWHM $\Gamma_t$ and amplitude $L_t$ at $\omega = 0$. [This is a slightly different normalization for the Lorentzian than that which appears in equation (51) or (58). This is necessary because of the normalization chosen in the fitting program.] The intensity which we want to
calculate is given by taking $\omega = 0$ in the sum of equations (129) and (130), and then using the appropriate value of $\Gamma_{res}$. The integral in equation (130) is easily evaluated in terms of the Voigt function shown in equations (75), (76), and (77), and the intensity calculated using the high energy-resolution experiment parameters is:

$$I_0(Q, \omega=0) = A(Q) + \frac{\sqrt{\pi} L_t(Q) \Gamma_t(Q)}{2K} \left[ i \frac{\Gamma_t(Q)}{2K} \right]$$  (131)

Finally, the relative contribution to the total scattering by the inelastic background for an arbitrary $\Gamma_{res}$ is given by:

$$C_0 = \frac{A(Q)}{A(Q) + \frac{\sqrt{\pi} L_t(Q) \Gamma_t(Q)}{2K} \left[ i \frac{\Gamma_t(Q)}{2K} \right]}.$$ (132)

We will evaluate equation (132) for $r_{res}^{hi} - k^{hi} = 0.0477$ meV and for $r_{res}^{low} - k^{low} = 0.397$ meV using the values of $A(Q)$, $L_t(Q)$, and $\Gamma_t(Q)$ shown for our samples in Table 5. Since we want to estimate the inelastic background intensity for samples which consist of hydrogenated molecules in D$_2$O, we have evaluated $C_0$ for the 10% Polyox in D$_2$O as shown in Table 15, because this is the only polymer in D$_2$O sample on which we collected high energy-resolution data. $C_0^{hi}$ ($C_0^{low}$) is the fraction of the scattering which is attributable to the inelastic background in a high (low) energy-resolution experiment.

From Table 15 we see that for this sample the contribution to the intensity from the inelastic background is quite large. For $Q = 2$ Å$^{-1}$, the contribution is greater than 37% in the high energy-resolution experiment and greater than 50% in the low energy-resolution
TABLE 15. Inelastic contribution to the intensity for 10% Polyox in D₂O

computed for two values of \( \Gamma_{\text{res}} \) using the parameters measured

in the high energy-resolution experiment.

<table>
<thead>
<tr>
<th>( Q (\text{A}^{-1}) )</th>
<th>( \frac{V(1) \Gamma_{\text{L}}(Q)}{2k\hbar l} )</th>
<th>( c_0 )</th>
<th>( V(1) \Gamma_{\text{L}}(Q) )</th>
<th>( c_0^{\text{LOW}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.720</td>
<td>0.095</td>
<td>0.958</td>
<td>0.396</td>
</tr>
<tr>
<td>0.7</td>
<td>0.607</td>
<td>0.140</td>
<td>0.934</td>
<td>0.486</td>
</tr>
<tr>
<td>1.0</td>
<td>0.457</td>
<td>0.197</td>
<td>0.889</td>
<td>0.512</td>
</tr>
<tr>
<td>1.1</td>
<td>0.366</td>
<td>0.217</td>
<td>0.850</td>
<td>0.499</td>
</tr>
<tr>
<td>1.2</td>
<td>0.352</td>
<td>0.249</td>
<td>0.843</td>
<td>0.536</td>
</tr>
<tr>
<td>1.5</td>
<td>0.309</td>
<td>0.324</td>
<td>0.818</td>
<td>0.601</td>
</tr>
<tr>
<td>1.9</td>
<td>0.166</td>
<td>0.369</td>
<td>0.676</td>
<td>0.544</td>
</tr>
</tbody>
</table>

experiment. Both of these values are greater than the estimated

contribution from the phonon model above. We now want to see how this
type of analysis can lead to corrections for the values of \( \langle u^2 \rangle /3 \).

We have plotted the inelastic background parameter \( A(Q) \) from the

Figure 10. The inelastic background parameter \( A(Q) \) plotted versus \( Q^2 \)

from a 10% Polyox/D₂O solution.
high energy-resolution experiment on the 10% Polyox in D₂O solution in Figure 10. It is clear that the behavior is quite different from the $Q^2 \exp(-Q^2 \langle u^2 \rangle/3)$ behavior predicted in equation (93) for the one phonon inelastic background. In fact, a more reasonable approximation would be:

$$A(Q) = a_0 + b_0 Q^2, \quad a_0 > 0, \quad b_0 > 0.$$  \hspace{1cm} (133)

A least-squares fit of the data to equation (133) gives $a_0 = 84 \pm 2.0$ and $b_0 = 7.6 \pm 1.4$. This means that the inelastic background gives an additional contribution to the intensity:

$$I_0(Q,\omega=0) = I_0 e^{-Q^2 \langle u^2 \rangle/3} + a_0 + b_0 Q^2$$ \hspace{1cm} (134)

which we have fit with an effective Debye-Waller factor, $I_0' e^{-Q^2 \langle u^2 \rangle/3}$. Expanding the exponential in equation (134) gives:

$$I_0(Q,\omega=0) = I_0 (1 - Q^2 \langle u^2 \rangle/3 + \cdots) + a_0 + b_0 Q^2$$ \hspace{1cm} (135)

$$= (I_0 + a_0) - (I_0 - 3b_0/\langle u^2 \rangle) \cdot Q^2 \langle u^2 \rangle/3$$ \hspace{1cm} (136)

$$= I_0' \left[1 - \frac{I_0 - 3b_0/\langle u^2 \rangle}{I_0'} Q^2 \langle u^2 \rangle/3\right]$$ \hspace{1cm} (137)

$$= I_0' \exp\left[-\frac{I_0}{I_0'} Q^2 \langle u^2 \rangle/3 + Q^2 \frac{b_0}{I_0'}\right]$$ \hspace{1cm} (138)
where we have set \( I_0' = I_0 + a_0 \). Thus, the effective Debye-Waller factor is:

\[
\frac{\langle u^2 \rangle}{3} = \frac{I_0}{I_0'} \frac{\langle u^2 \rangle}{3} - \frac{b_0}{I_0'},
\]

(139)

For the 10% Polyox in \( D_2O \) solution, the values of \( a_0 \) and \( b_0 \) quoted earlier and the value of \( I_0 \) shown in Table 7 give \( b_0/I_0' = 0.044 \pm 0.008 \) \( \text{A}^2 \). This is the same size as the values given in Table 14 for \( -B_U \); therefore, equation (139) gives a correction of the proper sign and order to make the values of the true thermal amplitude, \( \langle u^2 \rangle/3 \), positive at all temperatures. Unfortunately, we do not know the temperature dependence of the terms in equation (139), and we do not have any physical model for the particular form of the inelastic background which we chose in equation (133). However, the form of equation (139) suggests that one can correct the values of \( \langle u^2 \rangle/3 \) by adding some constant term to the values predicted by equation (119); the smallest number we can add to equation (119) to make \( \langle u^2 \rangle/3 \) positive at all temperatures is \( -B_U \). Therefore, the corrected values of \( \langle u^2 \rangle/3 \) are:

\[
\langle u^2 \rangle_{\text{corr}}/3 = \langle u^2 \rangle/3 + (-B_U) = A_U T + B_U + (-B_U)
\]

(140)

\[
\langle u^2 \rangle_{\text{corr}}/3 = A_U T
\]

(141)

using the values of \( A_U \) given in Tables 12 and 14. This is a purely empirical correction to the values of \( \langle u^2 \rangle/3 \), but we will use this correction later to compare our values with other values.
2. **The temperature dependence of \( \langle U^2 \rangle /3 \).**

We have shown that the values of \( \langle U^2 \rangle /3 \) have a temperature dependence given by:

\[
\langle U^2 \rangle /3 = A_u T + B_u
\]  

(119)

which must be corrected according to equations (127) or (141) to obtain the true thermal vibrational amplitude, \( \langle u^2 \rangle /3 \). These corrections are necessary because of the inelastic background. This is easily seen to be the correct temperature dependence if one considers that the Debye-Waller factor is primarily given by the vibrational motion of the scatterers. Then, by the energy equipartition principle, we have:

\[
\frac{1}{2} K_s \langle u^2 \rangle = \frac{3}{2} k_B T
\]  

(142)

where \( K_s \) is an effective spring constant for the forces which hold the hydrogen atoms in place. We expect this temperature dependence to hold down to the temperature at which zero-point motion occurs, at which point the values of \( \langle u^2 \rangle /3 \) should be constant in temperature. (The fact that equation (142) has no constant term also reinforces the correction given by equation (141).) We will calculate the effective spring constants, \( K_s \), from the values of \( A_u \) in Tables 12 and 14 and compare them to values for various chemical bonds as reported in the literature. The spring constant is given by:

\[
K_s = 1.381 A_u^{-1} \left( \text{dyne cm} \right)
\]  

(143)
where $A_u$ is in $A^2/K$ as in Tables 12 and 14. The calculated values of $K_s$ for our samples are shown in Table 16, and the values of $K_s$ for several types of chemical bonds are shown in Table 17.

**TABLE 16. Effective spring constants calculated from equation (143).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$A_u \cdot 10^4$</th>
<th>$K_s \cdot 10^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyoxy (8000) powder</td>
<td>2.98±1.09</td>
<td>0.046±0.002</td>
</tr>
<tr>
<td>Raw Trypsin powder</td>
<td>2.19±0.09</td>
<td>0.063±0.003</td>
</tr>
<tr>
<td>Treated Trypsin powder</td>
<td>2.50±0.04</td>
<td>0.055±0.008</td>
</tr>
<tr>
<td>Polyoxy (WSR-205)/D$_2$O</td>
<td>4.06±0.50</td>
<td>0.034±0.004</td>
</tr>
<tr>
<td>Polyoxy (8000)/D$_2$O</td>
<td>5.0±0.1</td>
<td>0.028±0.006</td>
</tr>
<tr>
<td>Raw Trypsin/D$_2$O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;275K</td>
<td>4.7±1.8</td>
<td>0.029±0.011</td>
</tr>
<tr>
<td>T&gt;275K</td>
<td>1.89±0.8</td>
<td>0.074±0.003</td>
</tr>
<tr>
<td>Treated Trypsin/D$_2$O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;275K</td>
<td>5.65±0.66</td>
<td>0.024±0.003</td>
</tr>
<tr>
<td>T&gt;275K</td>
<td>2.20±0.25</td>
<td>0.063±0.007</td>
</tr>
</tbody>
</table>

**TABLE 17. Effective spring constants for various bonds.**

<table>
<thead>
<tr>
<th>Chemical bond</th>
<th>Motion</th>
<th>$K_s \cdot 10^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Gas)$_{142}$: O-H</td>
<td>Stretch</td>
<td>8.454</td>
</tr>
<tr>
<td>Water (Gas)$_{142}$: H-O-H</td>
<td>Angle bend</td>
<td>0.761</td>
</tr>
<tr>
<td>Water (Ice-I)$_{142}$: O-H••O</td>
<td>Stretch</td>
<td>0.17 - 0.19</td>
</tr>
<tr>
<td>Water (Ice-I)$_{142}$: H-O-H</td>
<td>Angle bend</td>
<td>0.49 - 0.56</td>
</tr>
<tr>
<td>Water (Ice-I)$_{142}$: O-H••O</td>
<td>Angle bend</td>
<td>0.085 - 0.095</td>
</tr>
<tr>
<td>O-H (Gas)$_{143}$</td>
<td>Stretch</td>
<td>7.746</td>
</tr>
<tr>
<td>C=O (Gas)$_{143}$</td>
<td>Stretch</td>
<td>19.02</td>
</tr>
<tr>
<td>H-H (Gas)$_{143}$</td>
<td>Stretch</td>
<td>5.706</td>
</tr>
<tr>
<td>C-C (Gas)$_{143}$</td>
<td>Stretch</td>
<td>12.16</td>
</tr>
</tbody>
</table>

Note: •• represents a hydrogen bond. - represents a covalent bond.
We see that the calculated spring constants in Table 16 are much smaller than all but the O-H⋯O angle bending force constants. Thus, one may conclude that there is another contribution to the Debye-Waller factor temperature dependence other than vibrational motion, or that the motion is distributed over groups of N atoms so that the effective spring constant is $-K_{s}/N$.

There may be other contributions, besides vibrational motion, to the Debye-Waller factor. For example in a crystalline protein sample, Frauenfelder, et al.,$^{41}$ write the mean-square displacement of an atom as determined from X-ray scattering as:

$$<u^{2}>_{\text{eff}} = <u^{2}>_{1d} + <u^{2}>_{c} + <u^{2}>_{d} + <u^{2}>_{v} \tag{144}$$

Some of the terms in equation (144) may not be relevant to our Polyox samples, because in solution, the Polyox is a very flexible random coil polymer. This is in contrast to the protein in solution which has a distinct folded structure because of bonding between specific segments of the protein. We will now discuss the terms in equation (144) to show the significance of each one and its probable temperature dependence.

The term $<u^{2}>_{1d}$, which represents the smearing of the atomic position due to lattice disorders, is not relevant for incoherent neutron scattering since each scatterer contributes to the signal independently of the others. (This is also true in Mössbauer studies.$^{55}$)
The term \( <u^2>_c \) represents the contribution to the mean-square displacement due to transitions between conformational substates in a protein and probably does not affect \( <u^2>_{eff} \) in our Polyox samples. The concept of conformational substates has arisen in the study of protein dynamics and refers to the large number of slightly different configurations which are possible in a protein when it is folded into its equilibrium configuration. These substates are separated from one another by potential barriers which arise from the interactions between adjacent atoms and which depend on the details of particular bond angles and lengths. One expects that at some temperature, \( T_r \), there is a transition from rapid movement between conformational substates to a state where this motion is stopped because the potential barriers are too high. Thus, above \( T_r \), \( <u^2>_c \) is an increasing function of \( T \), and below \( T_r \), \( <u^2>_c \) is constant. If the macromolecule is in a solution which freezes at \( T_f \), there may be a change in the number of conformations available to the molecule because of the rigid structure of the lattice in which the molecule is imbedded. Thus, in the frozen solution, \( <u^2>_c \) may be slightly reduced from the value in the liquid state, and the slope of the \( <u^2>_c \) versus \( T \) curve might change.

Translational and rotational diffusion contribute to \( <u^2>_d \) in both polymer and protein solutions. For a molecule in dilute solution, this term should have a temperature dependence proportional to the diffusion coefficient of the molecule: \( D_{molecule} \sim \frac{T}{\eta} \sim D_{solvent} \), where \( \eta \) is the solvent viscosity.\(^{41, 78, 144-146}\) Above the freezing point of water or in supercooled water, the diffusion coefficient of water has been shown to have a temperature dependence given by:\(^{144, 147}\)
\[ D(T) = D_0 \exp \left( -\frac{E_a}{RT} \right) \]  \hspace{1cm} (145)

with a temperature dependent activation energy, \( E_a \), between 4 to 5 kocal/mole for temperatures between 273K and 300K. Expanding equation (145) around the freezing point of water, \( T_f \), gives:

\[ D(T) = D' \left[ 1 + (T-T_f) \frac{E_a}{RT_f^2} + \ldots \right] \]  \hspace{1cm} (146)

where \( D' \) is the diffusion coefficient at \( T_f \). This shows that \( \langle u^2 \rangle_d \) is an increasing function of temperature above \( T_f \). Below \( T_f \), the translational diffusion coefficient is reduced by a few orders of magnitude so that the contribution to \( \langle u^2 \rangle_d \) would be essentially zero. The rotational diffusion of molecular solutes will also be influenced by the freezing of the water and will have a temperature dependence like that for translational diffusion.

The vibrational contribution to the mean-square displacement, \( \langle u^2 \rangle_v \), will have a temperature dependence \(-T\) for temperatures above \( T_D \), the Debye temperature, \( ^{11} \) as discussed previously. Below \( T_D \), the contribution is constant in temperature. For a dilute solution of globular macromolecules such as trypsin, one would not expect the slope to change much at the freezing point of the solvent because most of the protons are confined to the interior of the molecule and their average environment would not change very much. However, the protons near the surface of the molecule would have a different environment and thus would have a different \( \langle u^2 \rangle_v \). Thus, the average of \( \langle u^2 \rangle_v \) over the whole molecule would be changed when the solution freezes. For an
extended or unfolded macromolecule, such as Polyox, the situation is
different. If there is a layer of bound water on its surface, one
might not expect any change in \( u^2 \) at the freezing point because the
hydration water surrounding the polymer is already in an ordered state
similar to ice so that there is no significant change in the
environment of the polymer protons. However, virtually all of the
protons in the molecule are exposed to the solvent and any small effect
on \( u^2 \) due to the freezing of the solvent would be apparent because
of the number of protons affected. Thus, we expect some change,
however small, in \( u^2 \) for both solutions when the solvent freezes.
Each of these contributions to the effective mean-square displacement
is sketched schematically in Figure 11.

Unfortunately, it is not possible to separate the contributions to
\( u^2 \) in our experiment. This same problem occurs in the X-ray and
Mössbauer experiments.\(^{41, 55}\) The problem is that the contributions all
have the same form of temperature dependence, and none of the
inflections in \( u^2 \) discussed earlier are observed with the
exception of the discontinuity in \( u^2 \) at the freezing point of the
trypsin solution. Perhaps we did not go low enough in
temperature,\(^{148-149}\) although we expected to see some inflection because
of the results of Parak, \textit{et al.},\(^{55}\) who saw an inflection at 210K.

3. Discussion of the Polyox results.

It is difficult to verify the values we have measured for \( u^2 \)
since no directly comparable experiments have been found in the
literature. However, there are some indications that the values are
reasonable. The temperature dependence is generally what one expects,
as we have already discussed. We will now compare the value of
Figure 11. Contributions to the mean-square displacement: a) vibration, b) conformational fluctuations, and c) diffusion.

\( \langle u^2 \rangle_{\text{corr}}/3 \) computed using equation (141) and the data in Table 14 to the value we measured for the 20% solution of Polyox (WSR-205) using the high energy-resolution method at room temperature (~300K). This is the only polymer sample for which we have data from both techniques.
Equation (141) gives $<u^2>_{corr}/3 = 0.12 \pm 0.02 \ \text{Å}^2$ for the 20% solution of Polyox in D$_2$O at 300K. Table 18 lists this value and the values computed using equations (119) and (127). The high energy-resolution technique gives $<u^2>/3 = 0.18 \pm 0.02 \ \text{Å}^2$ at room temperature as reported in Table 7. These two values are close, but there is no overlap of the error bars. Perhaps the slope in equation (141) needs to be adjusted as equation (139) implies. If we use $<u^2>/3 = 0.18 \ \text{Å}^2$ as a valid data point at 300K and require a line to pass through the origin, the slope is $A_u^{corr} = 5.9 \times 10^{-4} \ \text{Å}^2/\text{K}$, which is well outside the error bars for the value of $A_u$ for this sample.

The temperature dependence of $<u^2>/3$ for the Polyox solutions does not seem to show an inflection at the freezing point of the solvent. This is not easily understood since we expect the vibrational and diffusive contributions to $<u^2>_{eff}$ to change in such a way as to give an inflection at the freezing point.

4. Discussion of the Trypsin results.

For the trypsin solutions, using the data in Table 14 and equation (141), we compute $<u^2>_{corr}/3$ to be $0.17 \pm 0.02 \ \text{Å}^2$ for the raw trypsin solution and $0.14 \pm 0.05 \ \text{Å}^2$ for the treated trypsin solution. This value

<table>
<thead>
<tr>
<th>Sample</th>
<th>$&lt;u^2&gt;/3$ from equation (119)</th>
<th>$&lt;u^2&gt;/3$ from equation (127)</th>
<th>$&lt;u^2&gt;_{corr}/3$ from equation (141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyox in D$_2$O</td>
<td>$0.099 \pm 0.017$</td>
<td>$0.110 \pm 0.017$</td>
<td>$0.122 \pm 0.015$</td>
</tr>
<tr>
<td>Raw Trypsin in D$_2$O</td>
<td>$0.081 \pm 0.027$</td>
<td>$0.092 \pm 0.027$</td>
<td>$0.170 \pm 0.020$</td>
</tr>
<tr>
<td>Treated Trypsin in D$_2$O</td>
<td>$0.074 \pm 0.074$</td>
<td>$0.085 \pm 0.074$</td>
<td>$0.142 \pm 0.054$</td>
</tr>
</tbody>
</table>
is listed in Table 18 along with the values computed using equations (119) and (127).

We can compare our results as shown in Table 18 to the theoretical simulation of Wong and McCammon\textsuperscript{21} which gives values of $<u^2>/3$ at 300K. The values of $<u^2>$ that they report are for the heavy atoms in the protein, not the hydrogens. Nevertheless, as an approximation we can use these values because the covalently bound hydrogens will move the same amount as the atoms to which they are attached.\textsuperscript{150}

Wong and McCammon\textsuperscript{21} report that the average value of $<u^2>/3$ over all heavy atoms in the protein is 0.15 Å$^2$ with no error bars listed. This seems to fall between the values corrected according to equation (141) for the raw and treated samples.

We treated one of the trypsin samples to remove labile protons; the hydrogens which are expected to be exchanged are the ones near the surface of the protein because those are the ones exposed to the solvent. Since the surface protons have been exchanged with deuterons to some extent, we expect the value of $<u^2>$ to be determined by the interior protons which have been shown to have reduced $<u^2>$.\textsuperscript{20-21} This is what our data indicates: the values of $<u^2>/3$ shown in Table 18 for the raw trypsin solution appear to be larger than the values for the treated trypsin solution although the error bars overlap.

The trypsin solutions display a discontinuity in the $<u^2>/3$ versus temperature graphs at the freezing point of the solvent, which we take to be 273K. As discussed earlier, this may be taken as being due to the reduction of the diffusion coefficient and vibrational and conformational degrees of freedom upon the freezing of the solvent.
5. Comparison of the trypsin and Polyox solution results.

The spring constants, $K_s$, and temperature coefficients, $A_u$, listed in Table 16 for the Polyox and trypsin solutions are quite similar. This is interesting in light of the contrasting nature of these two molecules in solution. However, the Debye-Waller factors, which determine $K_s$ and $A_u$, are determined by the high frequency motion of individual hydrogen atoms and thus may not be too sensitive to macromolecular details outside of the immediate neighborhoods of the hydrogen atoms.

The most obvious difference between the Polyox and trypsin solutions is the behavior at the freezing point of the solvent. The data seems to indicate that the trypsin solution has a change in the slope of the $\langle u^2 \rangle$ versus temperature curve and a discontinuity in $\langle u^2 \rangle$ at the freezing point of the solvent. The Polyox solution apparently does not display this behavior. If these features are borne out by further investigation, it becomes difficult to explain the difference. We rationalized that the trypsin solution behavior was due to changes in the conformational, vibrational and diffusive contributions to $\langle u^2 \rangle_{\text{eff}}$, and there is no apparent reason why the Polyox should not have similar changes in the vibrational and diffusive contributions to $\langle u^2 \rangle_{\text{eff}}$.

6. Summary of the low energy-resolution results.

In summary, we have been able to measure Debye-Waller factors for two polymeric samples in powders and D$_2$O solutions using a technique which greatly reduces the time for data collection. We have had to deal with several experimental problems such as the elimination of crystalline Bragg peaks from the signal and the contribution to the
signal from the D₂O solvent. Nevertheless, the <u²> data seems to have the right temperature dependence with the exception of two features: 1) We see no leveling off of the <u²> versus T curve at low temperatures, and 2) the extrapolated value of <u²> at T=0 is negative because of negative values of B_u. The absence of inflections in the <u²> versus temperature curve prevents separation of any of the possible contributions to the Debye-Waller factor. We have shown by two arguments that the inelastic background can be responsible for the negative values of B_u. These arguments gave corrections which bring the data more into line with our high energy-resolution data and with the computer simulations of Wong and McCammon²¹ at 300K.
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