RICE UNIVERSITY

Addition of a Magnetite Layer onto a Polysulfone Water Treatment Membrane to Enhance Virus Removal

by

Isabel Raciny

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE Master of Science

APPROVED, THESIS COMMITTEE:

[Signatures]

Pedro Alvarez, George R. Brown Professor of Engineering and Department Chair
Civil and Environmental Engineering

Qilin Li, Assistant Professor
Civil and Environmental Engineering

Mason Tomson, Professor
Civil and Environmental Engineering

HOUSTON, TEXAS
MAY 2011
ABSTRACT

Addition of a Magnetite Layer onto a Polysulfone Water Treatment Membrane to Enhance Virus Removal

by

Isabel Raciny

The applicability of low-pressure membranes systems in distributed (point of use) water treatment is hindered by, among other things, their inability to remove potentially harmful viruses and ions via size exclusion. According to the USEPA and the Safe Drinking Water Act, drinking water treatment processes must be designed for 4-log virus removal. Batch experiments using magnetite nanoparticle (nano-Fe$_3$O$_4$) suspensions and water filtration experiments with Polysulfone (PSf) membranes coated with nano-Fe$_3$O$_4$ were conducted to assess the removal of a model virus (bacteriophage MS2). The membranes were coated via a simple filtration protocol. Unmodified membranes were a poor adsorbent for MS2 bacteriophage with less than 0.5-log removal, whereas membranes coated with magnetite nanoparticles exhibited a removal efficiency exceeding 99.99% (4-log). Thus, a cartridge of PSf membranes coated with nano-Fe$_3$O$_4$ particles could be used to remove viruses from water. Such membranes showed negligible iron leaching into the filtrate, thus obviating concern about colored water. Further research is needed to reduce the loss of water flux caused by coating.
ACKNOWLEDGEMENTS

Many individuals made possible to accomplish the work presented in this thesis.

First, I want to thank God for my life and Fidan Sophia for the time that I have stolen from her.

I would like to thank my research advisor Dr Pedro Alvarez for his insights, support and motivation. Dr Qilin Li who offered me helpful advice and support.

Thanks Dr Mason Tomson for being part of the committee.

I am thankful to Katherine Zodrow and Dr Dong Li, co-author of the research article that resulted from the work presented in this thesis. In particular, thank Katherine for her guidance in the laboratory. I would also like to thank Mike Liga, Sen Wang and Bridgette Bennett for laboratory assistance. I thank all of the other members of the Alvarez Lab and the Civil and Environmental Engineering Department.

I would like to thank the Center for Biological and Environmental Nanotechnology at Rice University (funded by the National Science Foundation) for partially funding the project and the Fulbright/Colciencias/DNP Fellowship and Universidad de los Andes for my financial support.

Finally thank my family and friends I greatly appreciate their invaluable support.
# TABLE OF CONTENTS

Title Page i
Abstract ii
Acknowledgments iii
Table of Contents iv
List of Figures vi
List of Tables vi
List of Symbols and Abbreviations vii

**Chapter 1: Introduction**

1.1 Membrane Technology for Water Treatment 1
1.2 Background on Viruses 3
1.3 Nanotechnology and Water Treatment 4
  1.3.1 Metal and Metal Oxides Nanoparticles 5
  1.3.2 Antiviral Properties of Iron Oxides Particles 7

**Chapter 2: Materials and Methods**

2.1 Membrane Coating with nano-Fe₃O₄ 10
2.2 Membrane Characterization 11
2.3 Virus Analysis 11

**Chapter 3: Results and Discussion**

3.1 Virus Adsorption onto Magnetite Nanoparticles (Batch) Experiments 15
3.2 Membrane Characterization 16
3.3 Virus Adsorption onto Nanomagnetite-Coated (nFe₃O₄-PSf) Membranes 17
3.4 Analysis of Iron in the Filtrate and Implications for Long-term Performance

Chapter 4: Conclusions

References
LIST OF FIGURES

**Figure 1** Global Installations for Low Pressure Membranes by Application. 3

**Figure 2** Removal of MS2 by 1 g/L magnetite nanoparticles at pH 6 after 1 h incubation in different electrolyte solutions. 16

**Figure 3** Virus removal by the addition of nano-Fe₃O₄-PSf Membranes. 18

**Figure 4**

a) Removal of MS2 by nFe₃O₄-PSf Membranes. 20

b) MS2 breakthrough curve from flow-through experiments with nFe₃O₄-PSf Membranes.

LIST OF TABLES

**Table 1** Basic Properties of the PSf and nFe₃O₄-PSf membranes. 17

**Table 2** Virus removal by nFe₃O₄-PSf membranes with different rinsing methods. 22
## LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl₃</td>
<td>Aluminum chloride</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer, Emmet and Teller method</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>°C</td>
<td>Celsius degree</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
</tr>
<tr>
<td>DBPs</td>
<td>Disinfection-byproducts</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GFD</td>
<td>Gallons per square foot per day</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HNO₃</td>
<td>Nitric acid</td>
</tr>
<tr>
<td>HPM</td>
<td>High pressure membrane</td>
</tr>
<tr>
<td>IEP</td>
<td>Isoelectric Point</td>
</tr>
</tbody>
</table>
L  Liter
LMH  Liters per square meter per hour
LPM  Low pressure membrane
m²  Square meter
m³  Cubic meter
M  Molar concentration
MF  Microfiltration
mg  Miligram
min  Minute
mL  Mililiter
mm  Millimeter
mM  Millimolar concentration
mV  Millivolt
μg  Microgram
μL  Microliter
μm  Micrometer
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>nano-$Fe_3O_4$</td>
<td>Magnetite nanoparticles</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NF</td>
<td>Nanofiltration</td>
</tr>
<tr>
<td>n-$Fe_3O_4$-PSf</td>
<td>Nanomagnetite-coated polysulfone</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural Organic Matter</td>
</tr>
<tr>
<td>PFU</td>
<td>Plaque Forming Units</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PSf</td>
<td>Polysulfone</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse Osmosis</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>TMP</td>
<td>Transmembrane pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>UF</td>
<td>Ultrafiltration</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
</tbody>
</table>
CHAPTER 1:

INTRODUCTION

According to the World Health Organization, one-sixth (1.1 billion people) of the world’s population lacks access to adequate water supplies (WHO, 2007). An estimated 31 million people contract virus-related gastrointestinal illnesses each year, of which only about 9 million are food-related (Mead et al., 1999). Groundwater, previously thought to be naturally pristine, has been found to contain enteric viruses (transported from faulty septic tanks, landfills, fields treated with waste sludge, latrines, or contaminated waterways), even in confined aquifers (Borchardt et al., 2007). Enteric viruses have also been detected in treated drinking water (Ehlers et al., 2005; Keswick et al., 1984; Lee and Kim, 2002; Vivier et al., 2004) and recent studies indicated that enteric viruses were the leading causative agents of waterborne diseases in the USA and worldwide (Fong and Lipp, 2005; Griffin et al., 2003). Therefore, the presence of enteric viruses in drinking water sources is a growing public health concern necessitating an effective, simple removal technology.

1.1 Membrane Technology for Water Treatment

Membrane treatment processes are considered a promising innovative technology to meet the current and future water treatment regulations. The goal is to ensure proper removal of pathogens, ion-sized particles and organic matter, desalination, and wastewater treatment for reuse.

The main advantages of membrane processes are the production of high quality water without chemicals or utilities, and small footprint (Pronk et al., 2009).
As an alternative to conventional water treatment, the application of membrane technology in water has increased dramatically in the last decade (Furukawa, 2008). New developments in membrane technologies (e.g., materials) resulted in the decreasing of membrane costs and energy requirements (Churchhouse, 2000).

Membrane filtration technologies employed in water treatment are classified in microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The main mechanism to remove water contaminants is size exclusion. Based on the operating transmembrane pressure (TMP), membranes employed in water treatment can be classified as Low-pressure membranes (LPM) and High-pressure membranes (HPM). Typically LPMs operate at TMPs less than 1 to 2 bar. LPMs include microfiltration and ultrafiltration.

The use of low-pressure membranes is growing worldwide. Their application in drinking water treatment and wastewater reuse accounted for 82% of the total installed capacity of LPM systems (Figure 1) (Furukawa, 2008). Low-pressure membranes processes (MF and UF) are capable of removing suspended or colloidal particles. Generally, these membrane media are manufactured in hollow-fiber modules. The pore sizes of MF membranes range from 0.1 – 0.2 μm. For UF, pore size generally range from 0.01 – 0.05 μm or less. The small pore size ensures high log-removal of microbial pathogens such as protozoan parasites (e.g., Cryptosporidium and Giardia) and bacteria (Hagen, 1998). Significant virus removal can be achieved using UF since their size ranges from 20-200 nm. Some recent limitations with respect to bacterial retention are reported for MF membranes (Wang et al., 2007). Overall, the application of these technologies faces some limitations that include their inability to remove ionic species and nano-sized particles such as viruses.
1.2 Background on Viruses

Viral particles are small (20-200 nm in size) and enteric viruses (e.g., Norovirus, hepatitis A virus, Enterovirus), which are usually sized 20-30 nm diameter, are among the most difficult water-borne microorganisms to remove (Langlet et al., 2009). The virion of most enteric viruses consists of nucleic acid genome encapsulated by a capsid composed of proteins containing weak acid and base groups (e.g., carboxyl, sulfhydryl and amine groups) that are ionizable (Brown and Sobsey, 2009). In natural aquatic environments viruses are charged biocolloidal particles with the ability to adsorb to solid surfaces, which influences viral fate and transport. The factors controlling the adhesion kinetics of viruses including the type of viruses and the associated surface properties are pH, ionic strength, degree of water saturation in soil, and the presence or absence of interfering substances such as natural organic matter (NOM), which may either adsorb the viruses or compete with them for adsorption sites on a surface (Bitton et al., 1976;
The net charge of a virus depends on the pH of the medium and the surface chemistry of the virus. The isoelectric point (IEP) is specific to the individual virus type and strain. Typically viruses have IEP in the range of 3-7 (Dowd et al., 1998); thus, they may be either positively or negatively charged in natural waters (pH 4-9). In most cases, viruses are negatively charged and positively charged surfaces can adsorb and possibly inactivate them in aqueous systems based on electrostatic interactions.

1.3 Nanotechnology and Water Treatment

Conventional water treatments methods have some limitations such as (Brame and Alvarez, 2011):

- Large scale infrastructure and material required.
- Generation of harmful disinfection-byproducts (DBPs).
- Pathogens resistance to disinfectants.
- Over time the water quality standards are becoming stricter and new contaminants are emerging.
- Use of aging water distribution infrastructure.

Nanotechnology has been identified as a promissory alternative to face some challenges to provide safe drinking water. The potential impact areas for nanotechnology in water treatment include three categories: (i) Treatment and Remediation, (ii) Sensing and detection and (iii) Pollution prevention (Theron et al., 2008).
Due to their large superficial area and high reactivity, many natural and engineered nanomaterials have excellent properties as adsorbents, catalysts, antimicrobials and sensors. Some antimicrobial nanomaterials include chitosan, silver nanoparticles (nAg), photocatalytic TiO$_2$, fullerol, aqueous fullerene nanoparticles (nC60) and carbon nanotubes (CNT) (Li et al., 2008). The incorporation of this nanomaterials in conventional or new technologies for water treatment is an innovative approach that enhances disinfection avoiding the production of disinfection byproducts and ineffectiveness to disinfectant-resistant pathogens.

1.3.1 Metal and Metal Oxides Nanoparticles

Silver nanoparticles (nAg)

Although silver compounds have been well known for long time to exhibit high toxicity toward microorganisms (Liau et al., 1997), it was only recently that silver nanoparticles started to be studied as antimicrobial agents towards bacteria (Sondi and Sondi, 2004, Singh et al., 2008) and viruses (Elechiguerra et al., 2005, Morones et al., 2005). The results indicate a stronger biocide effect on Gram-positive than against Gram-negative bacteria. The bactericidal efficacy of Ag nanoparticles seems to be dependent on particle size and shape. Regarding size, small particles (<10 nm) were found to be most effective toward certain bacteria among particles with size between 1-100 nm (Morones et al., 2005). Similarly, nanoparticles ranging from 1 to 10 nm inhibit certain viruses from binding to their host cells (Elechiguerra et al., 2005). Concerning shape, truncated triangular plates displayed the best biocide activity when compared to spherical or rod-shaped nanoparticles, probably due to differences in their active surface area (Pal et al., 2007). The exact mechanism of the bactericide effect of nAg is still in debate, but some of the mechanisms proposed are the penetration of the bacterial cell wall, membrane damage through
formation of free radicals, and damaging interactions of silver nanoparticles with either sulphur-containing proteins or DNA (Theron et al., 2008).

**Titanium Dioxide (TiO₂)**

During the last two decades, abundant information has been collected regarding the photocatalytic inactivation of bacteria by TiO₂ (Wei et al., 1994; Watts et al., 1995; Kikuchi et al., 1997; Cho et al., 2005; Benabbou et al., 2007; Page et al., 2007). It has also been found that nano-sized TiO₂ can inactivate viruses such as MS2 bacteriophage (Cho et al., 2005) among others. Its mode of action involves generation of reactive oxygen species (ROS) under UVA light (Kikuchi et al., 1997), which causes oxidative stress in the microorganisms and leads to cell death. In order to improve light absorbance of TiO₂, doping with noble metals is currently explored. In particular, it was recently demonstrated that doping TiO₂ with silver caused a remarkable improvement in viral inactivation (Liga et al., 2011). A recent approach is to couple the photocatalytic microbial effect of TiO₂ with membrane separation technology. This photocatalysis-membrane coupling technology has shown not only to preserve the functions of the two separate processes but also some synergistic effects, although many challenges still need to be solved (Xiao et al., 2010).

**Zinc Oxide (ZnO)**

Like TiO₂, zinc oxide also exhibits strong UV light absorption. Likewise, it has shown high bactericide activity against a broad spectrum of microorganisms (Huang et al., 2008). However, the mechanism of its antimicrobial activity and the effect of nanoparticle size is still under study (Raghupathi et al., 2011, Li et al., 2011). The suggested bactericidal mechanisms include
generation of ROS, deposition of the nanoparticles on the surface of bacteria, accumulation of the nanoparticles in the cytoplasm or in the periplasmic region, and generation of Zn$^{2+}$ ions or Zn$^{2+}$ labile complexes. Zinc oxide nanoparticles have also been used to remove arsenic from water, even though bulk zinc oxide cannot absorb arsenic (Tiwari et al., 2008).

**Iron Oxides**

Several nano-sized iron containing minerals, including hematite (Fe$_2$O$_3$) and magnetite (Fe$_3$O$_4$), have been studied for adsorption processes in wastewater treatment. The small size of those nanoparticles, typically 2-3 orders of magnitude smaller than bacteria, provides a much larger surface area than ferric oxide typically used in water treatment. The magnetic iron oxide minerals are collectively known as ferrites. Various ferrites, including magnetite, were evaluated in batch modes for actinide and heavy metal removal in wastewater. Also, ferrites have been employed to enhance removal of cobalt and iron from simulated groundwater (Tiwari et al., 2008). More recently, iron oxides have found application in virus removal for drinking water, but that will be discussed in more detail in the following section.

**1.3.2 Antiviral Properties of Iron Oxides Particles**

Virus adsorption onto different solid surfaces such as hematite, clays, activated carbon, ceramic modified materials and iron oxides/hydroxide species is well documented. Nonpathogenic model viruses, such as bacteriophages MS2, PRD1 and phi X174, and the enteric virus *Rotavirus* have been used to investigate virus transport in different media. Viruses are known to adsorb onto iron oxides commonly present in soil and artificially incorporated into filtration media. Magnetite sand and hematite particles have been shown to be effective filtration media for poliovirus
removal (Moore et al., 1981). Ryan et al. (2002) also reported increased removal of PRD1 and MS2 in a sand column by coating quartz sand with iron oxides. Effluent analysis indicated that the viruses had attached strongly to the medium, the viruses had been inactivated (potentially by the strong attractive force between the capsid and ferric oxyhydroxides), and the remaining virus nucleic acids were released into the effluent. Chu et al. (2003) found that viruses are removed effectively in soils that contained iron oxides, and that the most influential environmental factors for virus removal in addition to the presence of iron were pH, NOM, metal oxides contents and soil saturation with water. Bitton et al. (1976) suggested that magnetite was a good adsorbent even at low concentrations (300 ppm) and considered various environmental factors affecting adsorption of poliovirus in water and wastewater onto magnetite. Rao et al. (1981) used magnetite in conjunction with pH adjustment (to pH 3) and 0.0005 M AlCl3 to effectively adsorb and concentrate poliovirus I for coagulation. High retention capacities for MS2 have been reported using magnetite treated with successive cycles of acid and alkali washing (Atherton and Bell, 1983).

NOM in particulate and dissolved form decreases the retention capacity of soil for MS2. Gutierrez et al. (2009) showed high removal of Rotavirus and MS2 by glass fiber coated with hematite nanoparticles in batch and flow-through experiments. However, virus adsorption decreased in the presence of NOM and bicarbonate ions. Modified media such as ceramics containing Fe and Al oxides can enhance the virus adsorption and inactivation through sorption processes (Brown and Sobsey, 2009).

Overall, these past studies show that incorporating iron oxides into water filtration systems could
enhance virus removal. Furthermore, manipulations to develop positive surface charges from the protonation of iron oxides (e.g., by decreasing pH) can facilitate electrostatic attraction of negatively charged viruses and enhance their removal efficiency. However, the potential of virus removal by incorporating iron oxides into polymeric low-pressure membranes has received limited attention, and little is known about the effect of water chemistry on this approach.

This study considers the incorporation of magnetite nanoparticles (nano-Fe₃O₄) into polymeric microfiltration membranes, creating a one-step treatment for virus removal. Two types of experiments were conducted at bench scale (1) Batch adsorption experiments using magnetite nanoparticles suspensions to assess virus adsorption capacity in the presence of common inorganic ions (Na⁺ and Ca²⁺) on virus adsorption capacity, and (2) Membrane filtration experiments using polysulfone membranes coated with nano-Fe₃O₄ to assess virus removal efficiency and iron leaching. Results suggest that nano-Fe₃O₄ coated membranes could potentially be used in point of use devices or small membranes systems for virus removal to avoid formation of harmful disinfection by products associated with the use of chemical disinfectants.
2.1 Membrane Coating with \textit{nano-Fe}_3\textit{O}_4

Nanomagnetite-coated polysulfone membranes (nFe$_3$O$_4$-PSf) were synthesized as follows. Samples of a commercial polysulfone membrane (0.2 µm mean pore size, 47 mm diameter, HT Tuffryn; Pall Co.) were cut to coupons of 25 mm in diameter and soaked in 100% ethanol solution for 10 minutes to fully wet the membrane. Then magnetite nanoparticles (Sigma-Aldrich, nanopowder <50 nm particle size, BET surface area > 60 m$^2$/g, ≥ 98% purity) were coated onto the membrane surface by filtering 3 mL of a nanomagnetite suspension in ethanol at a concentration of 1 g/L through the membrane at a flow rate of ~1 mL/min. This resulted in a total iron content of 3.9% by weight. Prior to filtration, the nanomagnetite suspension was sonicated for 5 minutes using a probe sonicator (Sonic Ruptor 250 Ultrasonic Homogenizer, Omni International; Kennesaw, GA) and for 10 minutes using a bath sonicator (Branson Ultrasonic 5510; Danbury, CT) to ensure homogeneous magnetite nanoparticle dispersion. The membranes without rinsing were then dried in the oven at 100 °C for 30 min and stored at 4 °C. To assess the capacity of the membrane to retain the nanomagnetite particles on the membrane surface and in membrane pores during typical microfiltration processes, the coated membrane samples were subject to two different rinsing protocols: superficial rinsing thoroughly with deionized (DI) water for 5 min and/or transversal rinsing by filtering 30 mL of DI water through the membrane for 10 min. These two rinsing protocols simulate the hydraulic condition encountered during cross-flow and dead-end filtration, respectively.

\footnote{The information presented here concerning the addition of nanomagnetite into a polysulfone membranes has been accepted for publication in I. Raciny, K. R. Zodrow, D. Li, Q. Li, and P. J. J. Alvarez. Addition of a Magnetite Layer onto a Polysulfone Water Treatment Membrane to Enhance Virus Removal. \textit{Water Science and Technology}. (In press).}
2.2 Membrane Characterization

*Permeability and Contact Angle*. Membrane permeability was determined by measuring the deionized water flux at room temperature in an Amicon Stirred cell over a working pressure range of 5-25 psi. The flow was measured using a digital scale that monitors cumulative permeate volume as a function of time. Membrane hydrophobicity was assessed by sessile drop contact angle measurement of DI water using a contact angle analyzer (DROPImage Standard).

*Iron Concentration in the Permeate*. Iron leaching from the membrane was evaluated by analyzing the effluent (permeate) for total iron concentration using inductively-coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 4300DV, Norwalk, CT). Samples (4 mL) were preserved with 1% HNO₃ prior to analysis.

2.3 Virus Analysis

*Preparation and Quantification of MS2*. Bacteriophage MS2 (ATCC#15597-B1) was used as a model waterborne virus. MS2 has capsid properties similar to those of poliovirus (Badireddy et al., 2007) and is commonly used as a surrogate to evaluate human enteric virus removal (You et al., 2005). MS2 is a non-enveloped icosahedral single-strand RNA coliphage, with a diameter of 26.0-26.6 nm (VanDuin, 1988) and an isoelectric point of 2.2-3.9 (Gutierrez et al., 2009; Yuan et al., 2008; Zerda et al., 1985). A low isoelectric point indicates a high net negative charge on the virus surface at typical pH values of natural water. Langlet et al., (2009) discussed the physico-chemical characteristics of MS2 phage, identifying it as a worst-case scenario for the evaluation
of virus removal by membrane filtration (i.e., (i) small size, (ii) high negative surface charge and (iii) high degree of hydrophobicity).

MS2 was propagated according to the method described by Zhu et al. (2005). To propagate MS2, the bacteriophage (100 μL) was incubated with 100 μL of its *Escherichia coli* host (ATCC 15597) with a concentration of $4 \times 10^8$ CFU/mL for 10 min in 900 μL 0.1 M bicarbonate buffer (pH 8.3). Then, warm tryptic soy soft agar was added to the suspension and the mixture was deposited onto a Luria-Bertani agar plate using the agar-overlay technique (Kennedy et al., 1986). After incubation overnight at 37 °C, the viruses were removed from the plate with bicarbonate buffer. Approximately 5 mL of the viral suspension was added to the plate and left to incubate for 10 minutes. This suspension was then removed and centrifuged at 5,000 rpm for 1 min, and the supernatant was filtered through a 0.2 μm polyethersulfone filter. The resulting viral suspension was stored at 4 °C until use. The viral stock concentration was determined by the standard Plaque Forming Units (PFU) assay (ISO-10705-1). Viruses were detected by the formation of clear zones (plaques) on the bacterial mat. Dilutions exhibiting 20-300 plaques per plate were considered for MS2 enumeration. All virus assays were performed in duplicate, and the virus concentration was reported by averaging the number of plaques from two replicate plates. Removal was calculated as logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.

*Virus Adsorption onto Magnetite Nanoparticles.* Magnetite nanoparticles for batch adsorption experiments were purchased from READE advanced materials, Reno, NV. The nominal size range of the nanoparticles was from 20 nm to 30 nm. Surface area of the magnetite nanoparticles
was determined by BET surface analyzer to be 69.4 m$^2$/g. Suspensions of magnetite at different concentrations were prepared by adding different amounts of magnetite powder to 15 mL of 100 mg/L background electrolyte solution to a final concentration of 1 g/L. The pH of the suspension was then adjusted to pH 6 by adding 0.034 M of HCl or NaOH. The suspensions were ultrasonicated for 30 s using a bath sonicator (Branson Ultrasonic 5510; Danbury, CT) immediately prior to the adsorption experiments. To assess electrostatic attraction as a potential virus removal mechanism, the zeta potential of the magnetite nanoparticles in all test solutions was measured using a ZetaSizer Nano ZS (Malvern, Inc., Southborough, MA). The magnetite nanoparticles were found to be positively charged with the surface zeta potential in the range of +12.5 to +16 mV, suggesting electrostatic attraction between the magnetite nanoparticles and the negatively-charged MS2.

The MS2 stock was diluted into the magnetite suspensions to a final concentration of $10^6$ PFU/mL in 20 mL glass vials. These vials were shaken at 250 rpm at room temperature for 1 h. At predetermined time intervals, 1 mL samples were taken from the suspension and placed into a 1.5 mL vial. A horseshoe magnet was placed under the vial to separate the magnetite nanoparticles from the aqueous phase. The supernatant was then serially diluted, and the virus titer quantified using the agar overlay technique. Control experiments were conducted simultaneously using four different buffer solutions at pH 6: 1.7 mM NaCl+1 mM CaCl$_2$, 1.7 mM NaCl+2 mM CaCl$_2$, 3 mM NaCl or 4 mM NaCl.

*Virus Adsorption onto Magnetite-PSf Membranes.* Membrane filtration experiments were conducted to evaluate the MS2 adsorption capacity of the polysulfone MF membranes coated
with magnetite nanoparticles (nFe$_3$O$_4$-PSf). For every test, 3 mL of viral suspension in 0.1 M bicarbonate buffer (adjusted to pH 8.3) were used. The viral suspension with a concentration between $10^6$ and $10^7$ PFU/mL was filtered at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter, corresponding to a volumetric flux of $3\times10^{-3}$ m$^3$/m$^2$-min. Prior to each test the membrane sample was rinsed superficially and transversally by filtering DI water through the membrane. Samples of viral solution were taken from the feed (influent) and permeate (effluent) streams, and were subsequently serial-diluted according to the protocol described by Zodrow et al. (2009) and quantified by the PFU method. Each filtration experiment was carried out at least in duplicates. Control experiments for MS2 filtration on non-coated polysulfone membranes were performed to provide a baseline for virus removal by the PSf membrane alone.

A continuous flow experiment was also conducted to evaluate virus breakthrough at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter (volumetric flux of $3\times10^{-3}$ m$^3$/m$^2$-min). About 20 mL of viral suspension was filtered continuously through the membrane, and 3 mL permeate samples were collected. Virus removal was calculated as logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.
CHAPTER 3:
RESULTS AND DISCUSSION

3.1 Virus Adsorption onto Magnetite Nanoparticles (Batch) Experiments

The effect of the divalent Ca\(^{2+}\) versus monovalent Na\(^{+}\) cations on MS2 removal was compared (at pH 6) to investigate the effect of common inorganic cations on virus adsorption. The effect of ionic strength was also investigated by varying the salt concentration. Increasing the ionic strength with NaCl from 3 to 4 mM decreased removal (Figure 2). This is likely due to the greater charge screening effect at higher ionic strength, and consequently reduced electrostatic attraction between the negatively charged viruses and the positively charged magnetite nanoparticles. The removal of MS2 by 1 g/L magnetite nanoparticles increased when the divalent calcium ion Ca\(^{2+}\) was present. Ca\(^{2+}\) was more effective than Na\(^{+}\) in promoting virus removal (i.e., 2.7-log removal of MS2 was achieved in the presence of Ca\(^{2+}\) compared to less than 0.5-log removal in the presence of Na\(^{+}\) alone). This could be attributed to two factors: (1) Ca\(^{2+}\) promotes virus coagulation to form complexes, and (2) A small number of negatively charged sites exist on the overall positively charged magnetite surface. Ca\(^{2+}\) forms ionic bridges between the few negative charge sites on magnetite surface and those on MS2 capsid.

These results corroborate previous studies reporting that divalent cations enhanced the deposition of MS2 to NOM-coated silica surface and silica (Pham et al., 2009). Apparently, Ca\(^{2+}\) promotes electrostatic attraction due to its tendency to form complexes on the adsorbent surface, which bind to negatively charged carboxylate groups on the viral capsid proteins.
Figure 2. Removal of MS2 by 1 g/L magnetite nanoparticles at pH 6 after 1 h incubation in different electrolyte solutions. Initial virus concentration was $10^6$ PFU/mL.

3.2 Membrane Characterization

Polysulfone membranes coated with ~3 mg of nanomagnetite ($n\text{Fe}_3\text{O}_4$-PSf) had permeability three times lower than the control membrane (PSf) without $n\text{Fe}_3\text{O}_4$ (Table 1). The decrease in membrane permeability due to the hydraulic resistance imposed by the nanomagnetite coating layer represents a concern on the higher energy requirement for membrane filtration. On the other hand, the $n\text{Fe}_3\text{O}_4$ membrane was slightly more hydrophilic than the control sample with a contact angle 7% smaller than that of the control PSf membrane. This decrease in hydrophobicity has potential benefits in preventing membrane fouling (Cheryan, 1998).
Table 1. Basic Properties of the PSf and nFe₃O₄-PSf membranes. (Values presented as average ± range; n = 2)

<table>
<thead>
<tr>
<th></th>
<th>PSf</th>
<th>nFe₃O₄-PSf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability (LMH/psi)</td>
<td>222 ± 6.4</td>
<td>70 ± 5.1</td>
</tr>
<tr>
<td>Contact Angle (°)</td>
<td>70 ± 6.8</td>
<td>65 ± 6.3</td>
</tr>
</tbody>
</table>

3.3 Virus Adsorption onto Nanomagnetite-Coated (nFe₃O₄-PSf) Membranes

Significant virus removal (> 99.99% with average virus log removal of 4.4 ± 0.5 (n=9)) was observed when 3 mL of viral suspension was filtered through membranes coated with magnetite nanoparticles, a dramatic improvement relative to the control membranes without magnetite, which only showed less than 0.5 log removal (Figure 3). The removal of virus by nano-Fe₃O₄-PSf membrane is a novel significant finding.
Two mechanisms of virus removal were considered (1) size exclusion through the nanomagnetite coating (potentially clogging pores or decreasing the pore size), and (2) electrostatic adsorption of the viruses to the magnetite nanoparticles. A continuous flow experiment was carried out to evaluate n-Fe3O4-PSf membrane performance for MS2 removal and to determine the main virus removal mechanism. A 4.5-log removal was initially observed (Figure 4a). The MS2 breakthrough curve is presented in Figure 4b. The allowable breakthrough concentration (representing 4-log virus removal) was reached after 18 mL of filtrate was collected. The decreasing removal efficiency and increasing effluent virus concentration with increasing
cumulative filtrate volume suggests that adsorption likely via electrostatic interaction instead of size exclusion is the major removal mechanism. Adsorptive removal efficiency decreased as more and more adsorption sites were occupied. Size exclusion, which would result in a stable or increasing (due to pore blockage) removal, did not seem to be the predominant mechanism.

The permeate volume at breakthrough corresponds to approximately 25 min of filtration time at a typical permeate flux of 50 gallons per square foot per day (GFD), suggesting that the n-Fe₃O₄ coating approach could be sustainable if the magnetite nanoparticles can be regenerated at each backwash by adjusting the solution chemistry (e.g., pH) of the backwash water. Adsorption capacity can be defined as the number of infectious virus particles (PFU) adsorbed per gram of magnetite nanoparticles in solution (Gutierrez et al., 2009). Accordingly, the adsorption capacity for MS2 in a 0.1 M bicarbonate buffer solution (pH 8.3) for 4.5-log (average Log removal of MS2 in our continuous flow experiment) was 3.3×10⁹ PFU/g.
Figure 4. a) Removal of MS2 by nFe$_3$O$_4$-PSf Membranes. Initial virus concentration $\sim$10$^7$ PFU/mL. b) MS2 breakthrough curve from flow-through experiments with nFe$_3$O$_4$-PSf Membranes. The 4-log removal USEPA requirement is depicted as a dotted line.
3.4 Analysis of Iron in the Filtrate and Implications for Long-term Performance

The average total Fe in the membrane permeate (after filtration of 3 mL MS2 suspension) was 10.6 ± 0.006 µg/L, corresponding to 0.00106% over the total estimated amount of nanomagnetite coated on the membrane (3 mg). The effluent iron concentration was significantly below the 0.3 mg/L secondary standard for drinking water, which was set to prevent aesthetic impacts on color and taste.

During the operation of a membrane system, the membrane surface and the pore wall is subject to hydraulic shear, which can potentially disturb the nanomagnetite coating layer and release magnetite nanoparticles. Therefore, superficial and transversal rinsing protocols were performed to remove loosely attached magnetite nanoparticles before filtration experiments. These two rinsing protocols simulate the hydraulic condition encountered in cross-flow and dead-end filtration, respectively. As shown in Table 2, MS2 removal after both rinsing protocols decreased compared to that without rinsing, suggesting the loss of some magnetite nanoparticles during the rinse. However, the rinsed membranes were still able to achieve greater than 4-log removal of MS2, indicating that an effective coating layer remained. These results suggest that the simple coating procedure used in this study may be effectively applied to industrial membrane units. Long term durability testing is needed to determine the lifetime of the coating.
**Table 2.** Virus removal by nFe$_3$O$_4$-PSf membranes with different rinsing methods.

<table>
<thead>
<tr>
<th>Rinsing Membrane Method</th>
<th>Log MS2 Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No rinse</td>
<td>6.0</td>
</tr>
<tr>
<td>Superficial</td>
<td>4.3</td>
</tr>
<tr>
<td>Superficial/Transversal</td>
<td>4.4 ± 0.5</td>
</tr>
</tbody>
</table>
CHAPTER 4:
CONCLUSIONS

In agreement with the literature, we showed that magnetite successfully removes viruses by adsorption. Furthermore, polysulfone membranes coated with magnetite nanoparticles were effective in removing bacteriophage MS2, potentially obviating the need for pre- or post-treatment to remove viruses in a membrane based system. Commercial polysulfone membranes are notable for their widespread application in water filtration. Cartridges of polysulfone membranes coated with nano-Fe₃O₄ particles may be an option to consider as a point-of-use devices and modification to existing membrane filtration processes to remove viruses from water. Advantages of nanomagnetite coating of polysulfone membranes include the simple coating protocol, avoidance of harmful disinfection byproducts, and negligible iron leaching into the filtrate. Due to the limited virus adsorption capacity of membranes prepared in this fashion, regeneration of the nanomagnetite material is necessary for this approach to be sustainable.

Although these results are promising, much research remains to be conducted to determine the feasibility of such membranes to treat different source waters. Key challenges include coating methods that lower the filtration energy requirement, and backwash processes (e.g., with a basic solution) to regenerate adsorption capacity.
REFERENCES


