Synthesis and Characterization of Novel Polyethers and Polydimethylsiloxanes for Use in Biomaterials

by

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Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Macromolecular Science and Engineering

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January 19, 2009
Blacksburg, Va

Keywords: Poly(ethylene oxide), Polydimethylsiloxane, Magnetite, Ionenes, ATRP
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Abstract

This dissertation focuses on the use of novel polyethers and polydimethylsiloxanes in the stabilization of magnetite nanoparticles as well as biomedical applications. The colloidal stabilities of magnetite nanoparticles coated with polyethers containing various functional endgroups were studied. Different variables (e.g. polymer loading, polyether molecular weight and type of functional anchor group) were investigated to determine their effect on the long-term physiological stability of the polyether magnetite complexes.

One-part PDMS-magnetite nanoparticle fluids were synthesized using a high shear process and magnetic separation techniques. These one-part fluids are unique in the fact that they do not require the addition of a non-functional PDMS oligomer solvent to generate a magnetic hydrophobic fluid. A series of PDMS-magnetite nanoparticle fluids containing different molecular weight stabilizers were synthesized. A magnetic separation study was performed to determine if PDMS molecular weight influences the magnetic separation profiles of the fluids.

Well-defined PDMS-\textit{b}-PtBA and PDMS-\textit{b}-poly(acrylic acid) copolymers were synthesized using living free radical techniques from novel PDMS precursors as well as PDMS-based ionenes with different hard segment groups.
Acknowledgements:

I would firstly like to thank all of my labmates, mentors and mentees in the Riffle Group over the years. This includes (in no particular order): Linda Harris, Kristen Wilson, Michael Zalich, Michael Vadala, Philip Huffstetler, John Boyd, William Miles (actually part of the Davis group), Shane Thompson, Nikorn Pothayee, Thompson Mefford, Tim Vadala, Christian Reinholz, Shannon Ball, Max Too!, Dr. Lin, Ragy Ragheb and Ryan Weyers. I could write a tome about our adventures; however I will just say that I have greatly enjoyed my interactions, both personal and professional (?), with everybody during my tenure in the research group. You all have been a revolving cast of characters that helped me keep my (in)sanity during the tedium of graduate school.

Other collaborators that deserve acknowledgement are the physicists at the University of Western Australia. Thanks to Rob Woodward, Matt Carroll, and Annette Tyler for their significant contributions to my dissertation and hospitality during my trip to Perth. Tim St. Pierre, you deserve special thanks, because you sir are in a class of your own. I look forward to our hanging out again sometime in the future. Thanks to my colleagues and professors in the Macromolecular Interfaces with Life Sciences (MILES) program for all of the help and opportunities over the years.

The most important person to acknowledge in this work is my advisor, Dr. Judy Riffle. Her positive impact on my life is incalculable. I could not imagine a better PhD advisor. I would also like to thank my committee members: Alan Esker, who always told me the brutal truths that I sometimes needed to here to grow as a scientist; Rick Davis, for his understanding nature and willingness to always help; James McGrath, a wonderfully well-rounded gentleman
and professor with a refined taste in wine and food; and J. P. Dailey who was always more a friend than a superior in all of the right ways.

On a personal level, I would like to thank my partner, Ane Johnson. Not only did you teach me how to take out trash regularly and iron my clothes, but also drastically impacted my view of the world for the better. I look forward to growing with you in the future for as long as you’ll have me. My brother, Christopher, also deserves thanks for being disinterested enough in what I do professionally to keep me grounded. Finally, thanks to my friend Christopher Martin for occasionally destroying my possessions in the name of art and reminding me of what little actually matters on the outside of this construct most of us blindly inhabit.

“I’ve been set free and I’ve been bound

Let me tell you people what I found

I saw my head laughing, rolling on the ground

And now I’m set free

I’m set free to find a new illusion”

-Lou Reed
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<th>Description</th>
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<tr>
<td>ATRP</td>
<td>Atom Transfer Radical Polymerization</td>
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<tr>
<td>D₃</td>
<td>1,1,3,3,5,5-Hexamethyloctasiloxane</td>
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<tr>
<td>DABCO</td>
<td>1,4-Diazabicyclo[2.2.2]octane</td>
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<td>Dimethylformamide</td>
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<td>DMSO</td>
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<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>T₉</td>
<td>Glass Transition Temperature</td>
</tr>
<tr>
<td>Tₘ</td>
<td>Melting Point Temperature</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric Analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
</tbody>
</table>
CHAPTER 1: Introduction

This dissertation focuses on the synthesis and characterization of novel polyethers and polydimethylsiloxanes for use in biomaterials. Biocompatible polymer-magnetite complexes have been a research focus for many years. Polyether and polydimethylsiloxane dispersion stabilizers have been developed as the coatings for the iron oxide nanoparticles. In addition, novel functionalizations of siloxanes and the synthesis of polydimethylsiloxane-based ionenes have been explored.

Cyclodextrins have the unique ability to form inclusions complexes with a wide range of hydrophobic drug molecules. Chapter 2 provides an overview of cyclodextrins and their role in drug delivery and polymer synthesis. Also, the controlled addition of 2-hydroxypropyl-β-cyclodextrin onto heterobifunctional polyethers will be described.

Previous work on polyether dispersion stabilizers for magnetite nanoparticles has produced magnetic complexes that are dispersible in aqueous media. However, a time-dependent colloidal instability in physiological media was observed for these complexes, limiting the potential applications for which these complexes may be used. Research in our laboratories has focused on studying how the different variables (e.g. polymer loading, polyether molecular weight and type of functional anchor group) affect colloidal stability of the polyether-magnetite complexes, with the goal of developing complexes for biomedical applications that have long-term stability in physiological media. Chapters 3 and 4 discuss the colloidal stabilities of magnetite nanoparticles coated with polyethers containing various functional endgroups. A family of vinylsilyl-terminated poly(ethylene oxide) and poly(propylene oxide-β-ethylene oxide) polymers were synthesized using novel 3-hydroxypropyltrivinylsilane and 3-hydroxypropyltrimethylvinylsilane initiators developed by Vadala et al (Figure 1.1). The
vinylsilyl endgroups of these polyethers were modified to contain carboxylic acid, ammonium and zwitterionic phosphonate anchor groups. Monodisperse magnetite nanoparticles were synthesized using a high-temperature method using Fe(acac)$_3$ adapted from Pinna et al.$^8$ In addition to the synthesis of polyether stabilizers and magnetite complexes, a discussion of the physiological stability of these complexes is provided in these chapters.

![Chemical structures of trivinylsilyl-terminated poly(ethylene oxide) (top) and trivinylsilyl-terminated poly(propylene oxide-b-ethylene oxide) (bottom).](image)

**Figure 1.1.** Chemical structures of trivinylsilyl-terminated poly(ethylene oxide) (top) and trivinylsilyl-terminated poly(propylene oxide-b-ethylene oxide) (bottom).

Our research has also focused on the synthesis poly(dimethylsiloxane)-stabilized magnetite complexes, resulting in hydrophobic magnetic fluids with potential biomedical applications (e.g. treatment of retinal detachment).$^{4,9-11}$ Previous work has described the synthesis of tricarboxylic acid-functional poly(dimethylsiloxane) (PDMS) stabilizers and the formation of PDMS-magnetite complexes. Chemical coprecipitation of iron chloride salts was used to synthesize the magnetite used in these materials. These PDMS-magnetite complexes were dispersed in non-functional PDMS oligomers to create hydrophobic magnetic fluids. Chapter 5 describes the synthesis of ‘neat’ one-part PDMS-magnetite nanoparticle fluids, comprised of only magnetite and the PDMS dispersion stabilizer (i.e. no PDMS carrier fluid). This was achieved by using high shear conditions during the magnetite synthesis and magnetic separation of the PDMS-magnetite complex. Chapter 5 will also discuss the magnetic separation behaviors of a series of
one-part fluids along with small angle neutron scattering characterization of a 3,100 g mol$^{-1}$ PDMS-magnetite complex.

The focus of Chapter 6 is the synthesis and characterization of well-defined monofunctional polydimethylsiloxanes. Using a dimethylvinyl-terminated PDMS precursor synthesized via living anionic polymerization of D$_3$, a procedure to introduce amino-functionality to one end of the siloxane is described. This monofunctional amine-terminated PDMS was then functionalized with 2-bromoisobutyryl bromide, yielding a PDMS macroinitiator for the atom transfer radical polymerization (ATRP) of tert-butyl acrylate. A series of PDMS-$b$-poly(tert-butylacrylate) (PDMS-$b$-PrBA) copolymers were synthesized via the living free radical technique followed by a mild deprotection procedure yielding PDMS-$b$-poly(acrylic acid) copolymers. **Figure 1.2** shows the chemical structures of the monofunctional polydimethylsiloxanes described in this chapter.
Figure 1.2. Chemical structures of monofunctional amine-terminated PDMS, monobromo-terminated PDMS, PDMS-\textit{b}-PrBA and PDMS-\textit{b}-poly(acrylic acid) (top to bottom).

Polymers containing charged ammonium groups in their backbone are referred to as ionenes. Novel, phase-separated PDMS-based ionenes were developed in our laboratory. Difunctional PDMS was modified with 6-bromohexanoyl chloride. The dibromoalkyl-terminated polydimethylsiloxanes were reacted in a 1:1 stoichiometric ratio with compounds containing two tertiary amine groups (e.g. 1,6-(N,N’-dimethylaminomethyl)benzene and 1,4-diazabicyclo[2.2.2]octane) in a Menschutkin reaction-based step-growth polymerization. Chapter 7 will discuss the synthesis and characterization of these PDMS-based ionene films containing the different hard segment groups.
2.1: Introduction

2.1.1: History

Cyclodextrins are cyclic oligosaccharides synthesized from the enzymatic degradation of starch. The three main natural cyclodextrins, α-, β-, and γ, contain six, seven, and eight glucosidic units, respectively. Cyclodextrins have a hydrophilic exterior and a hydrophobic interior cavity. Guest molecules can fit inside the cavity of the cyclodextrin host, forming what is referred to as an inclusion complex. The chemical structures of α-, β-, and γ cyclodextrin are shown in Figure 2.1.

![Chemical structures of α-, β-, and γ cyclodextrin.](image)

French scientist, A. Villiers, published the discovery of a material later known to be a cyclodextrin in 1891. In this publication, he described the isolation of about 3 g of a crystalline material from the digestion of 1000 g starch with Bacillus amylobacter. Villiers named this material ‘cellulosine’, and determined its composition to be (C₆H₁₀O₅)₂•3H₂O. The material was noted for its similarities to cellulose, namely resistance towards acid hydrolysis.
and lack of reducing properties. Scientists today believe that Villers formed a mixture of \( \alpha^- \) and \( \beta^- \)-cyclodextrin in these digestion procedures.\(^{13,16}\)

In 1903, an Austrian microbiologist Franz Schardinger studying bacteria pertaining to food poisoning, isolated two separate crystalline compounds during the digestion of potato starch. Schardinger determined these two compounds to be the ‘cellulosines’ that Villers had discovered twelve years earlier.\(^{13,16}\) He initially renamed these compounds as ‘crystalline dextrins’, but later changed the names to \( \alpha^- \) and \( \beta^- \)-dextrin. The major crystalline product of these digestion experiments was determined to be \( \alpha^- \)-dextrin. To distinguish between the two compounds, Schardinger reacted them with iodine. The dry \( \alpha^- \)-dextrin/iodine complex formed a greenish color and the dry \( \beta^- \)-dextrin/iodine complex formed a reddish-brown color.\(^{13,15,16,19}\) Until 1911, Schardinger continued to publish on cyclodextrins, discovering that the materials could be synthesized from several sources of starch and bacteria.\(^{13,16,20}\)

Freudenberg et al. synthesized \( \gamma^- \)-cyclodextrin in 1935. The authors later determined from hydrolysis and acetolysis techniques that the crystalline dextrins were ringed structures comprised of \( \alpha \)-1,4-glycosidic linkages. The crystalline cyclic dextrins were renamed ‘cyclodextrins’.\(^{13,16,21-23}\)

During the 1950’s, Cramer et al. researched the physical and chemical properties of \( \alpha^- \), \( \beta^- \), and \( \gamma^- \)-cyclodextrin, including cavity size, structure, and reactivity. It was reported that by forming inclusion complexes with cyclodextrin, the solubility and oxidative stability of certain compounds could be increased.\(^{13,15,16,24}\) The unique properties of inclusion complexation led to an increased interest in using cyclodextrins in drug formulations. However, research on cyclodextrin products for human use was stalled for over two decades when French et al. reported in 1957 that cyclodextrin was toxic in animal studies. In this publication, a small population of
rats fed a diet of β-cyclodextrin all died within a week.\textsuperscript{13,16,25} It has been postulated that the β-cyclodextrin French used in this study contained a significant amount of toxic organic impurity. In the decades following this claim, cyclodextrins were deemed safe for human consumption and can be readily found as ingredients in foods, drugs, and cosmetics.\textsuperscript{13,15,16,26-28}

In 1970, cyclodextrins were only available as rare fine chemicals. The price of β-cyclodextrin at this time was approximately $2000 US per kg, making large-scale industrial use infeasible.\textsuperscript{13-16,29} Production of cyclodextrins entailed treating starch with amylase from \textit{Bacillus macerans}, yielding a mixture of \textasciitilde{}60% α-cyclodextrin, \textasciitilde{}20% β-cyclodextrin, and \textasciitilde{}20% γ-cyclodextrin. The separation and purification of these materials was an intensive process, which was reflected in their price.\textsuperscript{13,15,16} Later in the decade, advancements in biotechnology led to drastic improvements in the production of cyclodextrins. New types of CGTase enzymes were engineered to increase the activity and selectivity of α-, β-, and γ-cyclodextrin production, leading to high purity materials suitable for pharmaceutical use.\textsuperscript{13,15,16}

Today, the price of β-cyclodextrin is about $5 US per kg with an annual output of approximately 10,000 tons.\textsuperscript{13,15} The three main cyclodextrins, as well as a large number of derivatives, are produced in large-scale and studied extensively for use as drug carriers. Advantages of using cyclodextrin in drug delivery include flexibility in cavity size, a chemical structure with many potential chemical modification sites, ability to preserve structural integrity degradable drug molecules, ability to control the release rate profile of complexed drugs, and low toxicity.\textsuperscript{13,15,30,31}
2.1.2: Cyclodextrin Properties

The three naturally occurring cyclodextrins, α-, β-, and γ-, are macrocyclic torus structures composed of glucopyranose units. The conical cylinder structure of these cyclic oligosaccharides contains a cavity lined with H3/H5 protons and lone electron pairs from the glycosidic oxygen atoms, creating interior hydrophobic character. Primary and secondary hydroxyl groups are oriented outward, affording a hydrophilic exterior on the cyclodextrin molecule (Figure 2.2). A larger cavity diameter is observed on the side of the molecule containing the secondary hydroxyl groups, because the free rotation of the primary hydroxyls decrease the effective cavity diameter on their side. A property overview of the three major cyclodextrins is shown in Table 2.1.

![Figure 2.2](image)

**Figure 2.2.** Structural features of cyclodextrin illustrating the hydrophilic exterior and hydrophobic interior cavity. From: *Cyclodextrins and Their Complexes*; Dodziuk, H., Ed.; Wiley-VCH: Weinheim, 2006.

Cyclodextrin’s structural features allow for the selective formation of inclusion complexes with a range of other molecules. This ability is also known as molecular recognition, or chiral recognition when dealing with enantiomeric compounds. Data has shown that cyclodextrins are flexible macrocycles, allowing for different modes of entrance into their cavities. Picking an appropriate host/guest combination can yield a very high selectivity. Derivatives of cyclodextrins
have been synthesized with the aim of increasing their complexing ability and selectivity with a specific drug.\textsuperscript{13-15,32,37}

<table>
<thead>
<tr>
<th>Property</th>
<th>α-Cyclodextrin</th>
<th>β-Cyclodextrin</th>
<th>γ-Cyclodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>972</td>
<td>1134</td>
<td>1296</td>
</tr>
<tr>
<td>Approximate inner cavity diameter (pm)</td>
<td>500</td>
<td>620</td>
<td>800</td>
</tr>
<tr>
<td>Approximate outer diameter (pm)</td>
<td>1460</td>
<td>1540</td>
<td>1750</td>
</tr>
<tr>
<td>Approximate volume of cavity ($10^6$ pm$^3$)</td>
<td>174</td>
<td>262</td>
<td>427</td>
</tr>
<tr>
<td>Solubility in water at 25$^\circ$C (g/100 mL)</td>
<td>14.5</td>
<td>1.85</td>
<td>23.2</td>
</tr>
<tr>
<td>Surface tension (MN/m)</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Melting temperature range (°C)</td>
<td>255-260</td>
<td>255-265</td>
<td>240-245</td>
</tr>
<tr>
<td>Crystal water content (wt.%)</td>
<td>10.2</td>
<td>13-15</td>
<td>8-18</td>
</tr>
<tr>
<td>Water molecules in cavity</td>
<td>6</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

Cyclodextrins have a lower aqueous solubility than their linear dextrin counterparts, likely due to the strong binding interactions of the crystalline cyclodextrin molecules.\textsuperscript{13,14,16,31,32,35} β-cyclodextrin, the most prevalent of the three materials, has the ability to form intramolecular hydrogen bonds, causing it to have the lowest water solubility (1.85 g/100 mL) of the three natural cyclodextrins. To resolve this issue, the hydroxyl groups on β-cyclodextrin can be chemically modified to increase aqueous solubility. Such water-soluble derivatives include, hydroxypropyl-β-cyclodextrin and sulphobutyl-ether-β-cyclodextrin.\textsuperscript{15,38,39}
Larger cyclodextrins have been synthesized and studied in recent years. δ-cyclodextrin, a nine-membered ring, and ε-cyclodextrin, a ten-membered ring, are two examples of larger cyclodextrins.\textsuperscript{32,40-42} In addition to an inability to solubilize smaller drug molecules, these larger cyclodextrins have been shown to be less stable than the three main natural compounds, rendering them ineffective for pharmaceutical applications.\textsuperscript{15,34,43}

2.1.3: Cyclodextrin Derivatives

Numerous cyclodextrin derivatives have been synthesized for a number of purposes, including, increasing aqueous solubility, increasing selectivity of a host/guest combination, or controlling the release rate and bioavailability of a drug. The three natural cyclodextrins contain 18 (α), 21 (β), and 24 (γ) hydroxyl groups that can be chemically modified.\textsuperscript{15,33,43} Modification reactions with cyclodextrin are governed by two important issues, the nucleophilicity of the hydroxyl groups at the C2-, C3-, and C4-positions, and the ability of the cyclodextrin to form an inclusion complex with the reagents used.\textsuperscript{15,44} Figure 2.3 shows the atom numbering of the glucosidic unit in a cyclodextrin molecule.\textsuperscript{15}

The primary hydroxyl groups at the C6 positions are the most nucleophilic and basic. Secondary hydroxyl groups at the C2 positions are the most acidic, while the hydroxyl groups at the C3 position are the most inaccessible.\textsuperscript{15,44,45} Electrophilic reagents will preferentially attack the C6 positions, while reactive reagents will attack all positions. Due to the differences in reactivity between the three types of hydroxyl groups, substitution reactions with cyclodextrins are not totally random, and in some cases can be controlled with success.\textsuperscript{13,15,44-47}
In cases where exact position and number of substituents are not important, water-soluble cyclodextrin derivatives are easily achieved through the random modification of hydroxyl groups to hydroxylpropyl, sulfopropyl, carboxymethyl, or silyl groups. Frömming et al. reported that substitution of hydroxyl groups with an alkyl group results in a drastic increase in aqueous solubility. In the case of β-cyclodextrin, solubility in water increases with the degree of methylation until ~66% of the hydroxyl groups have been substituted. This enhancement of aqueous solubility in the alkyl derivatives is due to the conversion of the native, crystalline cyclodextrin into an amorphous, isomeric material. 2-Hydroxypropyl-β-cyclodextrin, a common derivative, is synthesized by reacting β-cyclodextrin with propylene oxide in basic conditions, yielding an isomeric final product with an average degree of substitution. Degree of substitution is not only important for the aqueous solubility of the derivatives; it also affects the guest molecule’s access to the interior cavity. The degree of substitution of β-cyclodextrin derivatives in pharmaceutical applications is typically around 0.65 for 2-hydroxypropyl and 1.8 for randomly methylated. Table 2.2 provides the aqueous solubilities of natural cyclodextrins and randomly modified cyclodextrin derivatives.

In cases where exact numbers and positions of substituents on the cyclodextrin molecule are needed for a well-characterized material (i.e. use in polymer synthesis), mono-modification reactions can be employed. The most common mono-modification derivative at the C6 β-
cyclodextrin position is 6-tosyl-β-cyclodextrin.\textsuperscript{15,24,34,38,39} The synthesis of this derivative involves reacting β-cyclodextrin with tosyl chloride in alkaline aqueous conditions, producing high yield mono-tosylated β-cyclodextrin. This derivative precursor is valuable because nucleophiles can attack the electrophilic 6-position carbon, producing cyclodextrin derivatives with a wide range of functionalities. Such varied 6-position mono-modified derivatives produced from this precursor include, amino, alkylamine, azide, chloro, and iodo-functional cyclodextrins.\textsuperscript{15,34,50}


<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>Substitution*</th>
<th>MW (Da)</th>
<th>Solubility in water** (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCD</td>
<td>-</td>
<td>972</td>
<td>145</td>
</tr>
<tr>
<td>βCD</td>
<td>-</td>
<td>1135</td>
<td>18.5</td>
</tr>
<tr>
<td>HPβCD</td>
<td>0.65</td>
<td>1400</td>
<td>&gt;600</td>
</tr>
<tr>
<td>RMβCD</td>
<td>1.8</td>
<td>1312</td>
<td>&gt;500</td>
</tr>
<tr>
<td>γCD</td>
<td>-</td>
<td>1297</td>
<td>232</td>
</tr>
<tr>
<td>HPγCD</td>
<td>0.6</td>
<td>1576</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

*Average number of substituents per glucopyranose unit  
**Solubility in water at 25 °C

Mono-modification reactions at the C2 and C3 positions on cyclodextrin may also be achieved. 2-Tosyl-β-cyclodextrin was synthesized by deprotonating β-cyclodextrin with NaH
and then reacting it with \( p\)-tosyl-1,2,4-triazole.\textsuperscript{15,51} Literature has shown that this regioselective precursor can react with many different reagents to form derivatives. It has been reported that alkylation of 2-tosyl-\( \beta \)-cyclodextrin with diaminoalkanes followed by a subsequent reaction with N-hydroxysuccinimide produced cyclodextrin dimers.\textsuperscript{15,52} Chen et al. showed that a \( \beta \)-cyclodextrin-2-chitosan conjugate was obtained from the reaction of 2-tosyl-\( \beta \)-cyclodextrin with chitosan.\textsuperscript{15,53} Besides the mono-tosylation of the C2 position on cyclodextrin, C2 monosulfonation has been synthesized via the reaction of \( \gamma \)-cyclodextrin with sulfonil imidazole in the presence of molecular sieves.\textsuperscript{15,51} Mono-modification at the C3 position is less common due to its inaccessibility. Chiu et al. showed that 2,3-mannoepoxy-\( \beta \)-cyclodextrin, synthesized from the 2-tosyl-\( \beta \)-cyclodextrin precursor, could be attacked by a nucleophile at the C3 position.\textsuperscript{15,54,55} The subsequent ring-opening reaction would yield a mono-modification at the C3 position on cyclodextrin.

### 2.2 Cyclodextrin-Drug Inclusion Complexes

#### 2.2.1 Cyclodextrin Inclusion Complexes

Cyclodextrin cavities are rarely empty. If no guest molecule is present for complexation, there usually are solvent molecules in the cavity.\textsuperscript{15,54,56} Inclusion complexes are formed when at least one guest molecule is partially contained within the cyclodextrin cavity. Inclusion complexes can be formed both in solution, where a cosolvent or heating may be required, and in the solid state, through cogrinding.\textsuperscript{15,54,56} In aqueous solutions, the cyclodextrin cavity is filled with water molecules, which unfavorable due to the hydrophilic-hydrophilic interactions.\textsuperscript{13-15,57} Appropriate guest molecules (less polar than water) can substitute the water molecules in the cavity (Figure 2.4).\textsuperscript{13} Factors that contribute to the forces driving complexation include the
release of high-energy water from the cavity and guest stabilization by weak van der Waals interactions. Inclusion complexes formed in solution can be recovered as stable crystalline materials.\textsuperscript{13-15,52,57,58}

Depending on the fit inside the cyclodextrin cavity, guest molecules may form inclusion complexes with different stoichiometries. Host:guest ratios may be 1:1, 2:1, 1:2, 2:2, etc.\textsuperscript{13,15,39,57,59,60} For example, \textit{p}-nitrophenol:$\alpha$-cyclodextrin has a 1:1 stoichiometry, whereas \textit{C}_60:$\gamma$-cyclodextrin has a 1:2 stoichiometry.\textsuperscript{15} The physiochemical properties of the guest molecule are altered once complexed with cyclodextrin, especially the aqueous solubility. For example, Montassier et al. has shown that that aqueous solubility of tretinoin ($8\times10^{-3}$ mg/ml), the acidic form of Vitamin A, increases to $2.7\times10^3$ mg/ml after complexation with $\beta$-cyclodextrin.\textsuperscript{61} Other properties that are altered after complexation include the reactivity of the guest molecules, diffusion/volatility, and spectral information (UV, NMR, etc.).\textsuperscript{13,62}


When an inclusion complex is formed in solution, equilibrium between the dissociated species and the associated complex is established. Drug complexation with cyclodextrin is defined as $K_{\text{assoc}}$ (\textbf{Eq. 2.1}).\textsuperscript{14} \textit{[Drug]} and \textit{[CD]} represents the concentrations of the dissociated species, and \textit{[Drug-CD]} is the inclusion complex concentration. Due to a lack of covalent bond
formation in the guest-host complex, complexation with cyclodextrin is said to be a dynamic process.\textsuperscript{13-15} \( K_{\text{assoc}} \) differs with the type of cyclodextrin derivative and guest molecule. In addition, dissociation of the inclusion complex may increase with dilute concentrations and/or the presence of a biological membrane/matrix for which the drug/guest molecule has a greater affinity. Experimentally, NMR can be used to detect the formation of cyclodextrin inclusion complex with a guest molecule.\textsuperscript{13-15}

\[
[\text{Drug}] + [\text{CD}] \xrightleftharpoons{K_{\text{assoc}}}^{K_{\text{assoc}}}[\text{Drug-CD}] / [\text{Drug}][\text{CD}]
\]

\textbf{Eq. 2.1}

Many methods have been employed to increase the complexation efficiency of cyclodextrin with a drug. Researchers have shown that both ionization and salt formation of a drug can result in enhanced complexation with cyclodextrin.\textsuperscript{15,16,33} Other methods of complexation enhancement incorporate a third species in the inclusion complex, such as a water-soluble polymer or an organic acid/base, forming a ternary complex with the cyclodextrin/drug.\textsuperscript{15,16,63}

\subsection{2.2.2 Cyclodextrin Inclusion Complexes as Drug Carriers}

The unique properties of cyclodextrins have proven to be beneficial in the field of ophthalmology. For local drug administration from a topical eye drop solution to be successful, the drug ideally must have some solubility in aqueous media, as well as have enough hydrophobic character to penetrate the lipophilic membranes in the eye.\textsuperscript{16,64-66} Hydrophobic corticosteroids, such as dexamethasone, are commonly used in aqueous eye drops for the treatment of ocular inflammation, but have poor water solubility. Traditional approaches to address this problem have been to make suspensions or water-soluble acetate/phosphate esters of the steroid in eye
drop solution. However, the low aqueous solubility of the suspensions and the lack of hydrophobicity in the ester analogues have hindered the efficacy of these approaches.\textsuperscript{64,67}

Cyclodextrin/drug inclusion complexes have been investigated extensively for use in ocular therapies. Aqueous soluble complexes can be formed with the lipophilic drugs using cyclodextrins, which allows for increased drug delivery. \textbf{Figure 2.5} shows how 2-hydroxypropyl-β-cyclodextrin significantly increases dexamethasone solubility in water.\textsuperscript{64} In this experiment, Gavrilin et al. added different concentration cyclodextrin solutions to 50 mg of dexamethasone.\textsuperscript{64} After stirring for one day, the concentration of dexamethasone in the aqueous solution was determined by UV spectroscopy.

\textbf{Figure 2.5.} Effect of 2-hydroxypropyl-beta-cyclodextrin (HPCD) concentration on aqueous dexamethasone (DM) concentration. Adapted from: Gavrilin, M. V.; Kompantseva, E. V.; Gusova, B. A.; Ushakova, L. S.; Makarov, V. A.; Karpenya, L. I. \textit{Pharmaceutical Chemistry Journal (Translation of Khimiko-Farmatsevticheskii Zhurnal)} 2000, 33, 160-163.
Studies have shown that cyclodextrin acts as a suitable carrier in water for hydrophobic drug molecules.\textsuperscript{66} This allows for a greater number of drug molecules to reach the lipophilic membranes in the eye. Once the inclusion complex reaches the membrane surface, the drug dissociates from the cyclodextrin and penetrates the hydrophobic membrane.\textsuperscript{66,68} The free cyclodextrin remains in the aqueous solution. \textbf{Figure 2.6} shows a diagram of drug penetration into the eye from an aqueous cyclodextrin solution.\textsuperscript{66}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram}
\caption{Drug penetration through the cornea from an aqueous cyclodextrin eye drop solution. Adapted from: Loftsson, T.; Stefansson, E. \textit{Acta Ophthalmologica Scandinavica} 2002, 80, 144-150.}
\end{figure}

Cyclodextrin-steroid eye drop formulations have shown promising experimental results.\textsuperscript{66,67,69,70} Loftsson et al. tested a dexamethasone solution containing 2-hydroxypropyl-\(\beta\)-cyclodextrin against a dexamethasone alcohol suspension in rabbit studies.\textsuperscript{66} A single drop of each solution was applied to a rabbit’s eye, and aqueous humor samples where tested for dexamethasone concentrations at various times. The dexamethasone concentrations over time
from the sample containing cyclodextrin were significantly higher than the alcohol suspension (Figure 2.7).\textsuperscript{66}

Researchers also studied randomly methylated-β-cyclodextrin as a potential dexamethasone carrier in ocular therapies.\textsuperscript{16,68,71,72} After the application of eye drops in rabbits, the hydrophilic 2-hydroxypropyl-β-cyclodextrin/1.3% (w/v) dexamethasone solution yielded a steroid concentration of 320 ng/g aqueous humor and the lipophilic randomly methylated -β-cyclodextrin/1.5% (w/v) dexamethasone solution yielded a steroid concentration of 66 ng/g aqueous humor.\textsuperscript{16} Where both cyclodextrins increase steroid delivery, this study shows the importance of choosing a suitable cyclodextrin derivative for an application.

Saarinen-Savolainen et al. reported the cytotoxicity of ophthalmic drugs and cyclodextrins.\textsuperscript{73} An immortalized human corneal epithelial cell line (HCE) was used to test toxicity. MTT assay results of the exposed HCE lines showed that 2-hydroxypropyl-β-cyclodextrin induced little to no membrane damage and was deemed safe for exposure to the corneal epithelium.\textsuperscript{73}

\textbf{Figure 2.7.} Dexamethasone concentration over time in the aqueous humor after administration of the cyclodextrin solution (solid) and the alochol suspension (empty). From: Loftsson, T.; Stefansson, E. \textit{Acta Ophthalmologica Scandinavica} 2002, 80, 144-150.
Other fields are investigating cyclodextrin inclusion complexes as drug carriers. In one case, Srichana et al. studied γ-cyclodextrin and dimethyl-β-cyclodextrin for use in dry powder aerosol formulations containing salbutamol, a drug commonly used in inhalers. In a model study using a twin stage impinger, both cyclodextrin formulations showed a faster and more efficient drug release profile than the control formulations. In conclusion, it was shown that γ-cyclodextrin had a better delivery efficiency and a lower toxicity.

In the field of HIV research, β-cyclodextrin has been used in conjunction with Nelfinavir Mesylate (NM), an Anti-HIV drug, to study the inclusion complex properties. A 1:1 NM:β-cyclodextrin was prepared in the solid state using cogrinding techniques, and was confirmed during NMR characterization. The inclusion complex showed better dissolution rates in distilled water than NM itself. Intestinal absorption studies revealed a 2.13 absorption rate enhancement in the inclusion complex over the plain NM. The researchers concluded that β-cyclodextrin:NM complexes are desirable for their enhanced solubility and drug bioavailability, and may improve dosage regimens for patients suffered from HIV.

2.2.3 Cyclodextrin Drug Release Rates from Polymeric Delivery Systems

As stated before, the type of cyclodextrin and subsequent chemical modification affects the release rate of a drug from an inclusion complex. Furthermore, incorporation of cyclodextrin inclusion complexes into polymeric delivery systems as physical mixtures or covalently bound species allows for even greater control in the release rate of the drug. In this section, the factors that influence the drug release mechanism in polymeric systems will be reviewed.

Physical mixtures of polymers and inclusion complexes will be discussed first. Depending on whether a cyclodextrin increases or decreases the concentration of diffusible species in the polymeric matrix will change if an enhanced or retarded drug release rate is
observed. Bibby et al. described these mechanisms of drug release from polymeric systems based on data from reported literature.\textsuperscript{14}

In the first case, assuming there is an inclusion complex physically blended into a hydrated polymer matrix, drug release will be enhanced if the drug concentration is above saturation.\textsuperscript{14,65,66} Here, after $K_{\text{assoc}}$ equilibrium between the drug and cyclodextrin is established, solid drug will be present in the matrix. As the drug concentration is over-saturated, inclusion complex formation does not decrease the free drug concentration, creating a total drug release rate that is additive of free drug and inclusion complex diffusion (\textit{Figure 2.8}).\textsuperscript{14} It is important to note that free drug is capable of diffusing through the hydrated matrix at a faster rate than the inclusion complex, due to a lower MW.

Alternatively, if the drug concentration is below saturation, inclusion complex formation will decrease the free drug concentration in the hydrated matrix, retarding the total drug release rate.\textsuperscript{14,16,66}

\textbf{Figure 2.8.} Mechanism of drug release from a hydrated matrix containing a physically blended inclusion complex and free drug. From: Bibby, D. C., Davies, N.M., Tucker, I.G. \textit{Int. J. Pharm.} 2000, 197, 1-11.
Researchers synthesized crosslinked chitosan microspheres containing a 2-hydroxypropyl-β-cyclodextrin inclusion complex with nifedipine, a calcium channel blocker.\textsuperscript{14,78} Despite the fact that the cyclodextrin increased the solubility of the poorly soluble drug, release rate was decreased due to the low diffusivity of the inclusion complex. Besides decreasing the diffusivity of the inclusion complex in the polymeric matrix, choosing a cyclodextrin with poor water solubility will also retard drug release rate.

Physically mixed cyclodextrin inclusion complexes can enhance drug release rate by promoting channeling or the erosion of the polymeric matrix. In one study, a pellet composition of 5:90:5 microcrystalline cellulose:β-cyclodextrin:cortiosteroid showed fast drug release in phosphate buffer solution.\textsuperscript{14,79} As the concentration of β-cyclodextrin was decreased in the pellet, the drug release rate decreased. It was concluded that as the pellets came into contact with water, β-cyclodextrin dissolved, increasing their porosity and increasing the release rate of the drug.

Covalently bound and crosslinked cyclodextrin-polymer systems also affect drug release rate. These systems are typically synthesized by reacting cyclodextrin with pre-made polymers or by using a crosslinking agent (i.e. epichlorohydrin) on the cyclodextrin itself.\textsuperscript{14} With these systems, the cyclodextrin is immobilized within the polymer matrix. Szeman et al. reported that covalently bound cyclodextrin-polymer systems still have the capability of forming inclusion complexes with drugs.\textsuperscript{14,80} However it was noted that the $K_{\text{assoc}}$ in these systems was typically less than unbound cyclodextrin inclusion complexes. If a drug forms an inclusion complex with the immobilized cyclodextrin, the drug’s diffusivity through the matrix will be slowed significantly, retarding release rate.

García-González et al. synthesized hydrogels of poly(acrylic acid) crosslinked with β-cyclodextrin via heating at 90°C for several hours.\textsuperscript{14,81} The authors proposed a condensation
mechanism where the primary β-cyclodextrin hydroxyl groups reacted with the carboxylic acid groups on the poly(acrylic acid) forming ester crosslinks. It was found that the β-cyclodextrin concentration in the hydrogels affected both swelling and drug release rate. Hydrogel swelling and polymer mesh size decreased as β-cyclodextrin concentration (crosslink density) was increased. These factors were claimed to result in the retardation of drug release rate.

To note a relevant contention to this research, two groups, Blanco-Fuente et al. and Bibby et al., claim that the esterification of cyclodextrin with poly(acrylic acid) at the above reported conditions was unlikely. A more likely scenario they proposed was polymer acid anhydride formation. This was supported by a $^{13}$C NMR study that showed acid anhydride formation and no definitive evidence of β-cyclodextrin as a crosslinking agent in the hydrogels.

### 2.3 Cyclodextrins in Polymer Synthesis

Cyclodextrins have widespread applications in polymer synthesis. Research efforts aim to attach cyclodextrin side chains onto polymers, incorporate cyclodextrin into polymer backbones, and utilize cyclodextrin’s ability to solubilize hydrophobic monomers in aqueous media. Cyclodextrin mediated aqueous polymerizations have received positive attention, because it allows for substitution of traditional organic solvents for a cheaper and more environmentally conscious process. The majority of the literature on these aqueous mediated processes deals with free radical polymerizations. First, inclusion complexes are formed between the cyclodextrin molecule and the hydrophobic monomer in the solid state. The complexes are then redispersed in an aqueous medium in the presence of a free radical initiator. During propagation, the cyclodextrin slips off the polymer chain, leaving an insoluble polymer. Researchers have studied the cyclodextrin mediated aqueous polymerizations with many types of hydrophobic monomers, including acrylates, methacrylates, and styrenes.
Madison and Long used a randomly methylated β-cyclodextrin (MeCD) with a 1.8 degree of substitution to polymerize tert-butyl methacrylate, cyclohexyl methacrylate, and 2-ethylhexyl methacrylate in aqueous media. The hydrophobic monomer and MeCD were dissolved in chloroform and allowed to stir for one day. Chloroform was removed to isolate the inclusion complex product. Ritter et al. had earlier used a longer complexation time of 6 days in chloroform, however, 1H NMR showed that shorter times were sufficient to form the inclusion complex. Madison and Long determined that the molar ratio of monomer/MeCD ranged from 0.50/1.0 to 0.75/1.0, depending on the system, from TGA and 1H NMR characterization. The homopolymerizations of the complexes were carried out in deionized water (50°C) using potassium persulfate as the free radical initiator. After 24 h, the hydrophobic methacrylic polymer precipitated from solution (Scheme 2.1).

Characterization of the purified poly(alkyl methacrylate) polymers revealed trace amounts of MeCD. This evidence supports the proposed cyclodextrin dethreading mechanism during free radical propagation that was mentioned earlier. The poly(alkyl methacrylate)s synthesized via the aqueous mediated process produced materials with number average molecular weights ranging from 50,000 to 150,000 g mol⁻¹ and polydispersity indices (PDI) above 3.0. Table 2.3 contains molecular weight and PDI data for poly(tert-butylmethacrylate) synthesized under different conditions.
Bernhardt et al. researched the initial polymerization rates of poly(alkyl acrylate)s from MeCD complexes in aqueous media. The hydrophobic character of the alkyl acrylate side group was increased as follows: propyl, butyl, pentyl, hexyl. UV spectroscopic and HPLC techniques were used to measure the amount of unreacted monomer complex in solution, allowing for the generation of concentration-time curves. The authors reported that initial polymerization rates increased with the hydrophobic character of the complexed monomer (Fastest rate: hexyl side chain; Slowest rate: propyl side chain). It was proposed that the more hydrophobic monomer complexes had a higher local concentration at the radical chain end of the phase-separated propagating polymer.

Using a different approach, Storsberg et al. employed semi-continuous monomer feed conditions for the MeCD mediated aqueous polymerizations of styrene and methyl methacrylate monomers. Unlike batchwise polymerizations of this type, no precipitation or coagulation
occurred during propagation. As a result, high monomer conversion and lower PDIs (~2.0-2.2) are achieved using this approach. The semi-continuous procedure entails preparing MeCD aqueous solutions of different concentrations, adding the free radical initiator, and then slowly adding the hydrophobic monomer dropwise over several hours at 80°C. Stable latex particles of polystyrene and poly(methyl methacrylate) with low particle size distributions were recovered from these polymerizations. The authors postulate that the high initial ratio of MeCD to monomer allows for rapid complexation, creating sites for micellar nucleation.92


<table>
<thead>
<tr>
<th>Polymer</th>
<th>Conditions</th>
<th>Temp (°C)</th>
<th>$M_n$</th>
<th>$M_w/M_n$</th>
<th>$T_g$ (°C)</th>
<th>% Yield</th>
</tr>
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<tr>
<td>Poly(tBuMA)1</td>
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<td>137,000</td>
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<td>107</td>
<td>77</td>
</tr>
<tr>
<td>Poly(tBuMA)2</td>
<td>B</td>
<td>50</td>
<td>90,000</td>
<td>3.69</td>
<td>120</td>
<td>85</td>
</tr>
<tr>
<td>Poly(tBuMA)3</td>
<td>A</td>
<td>60</td>
<td>48,000</td>
<td>3.62</td>
<td>125</td>
<td>65</td>
</tr>
</tbody>
</table>

Glöckner, Metz, and Ritter evaluated the use of chain transfer agents to control the MeCD mediated aqueous polymerizations.87,93 Methyl methacrylate and styrene monomers were investigated in the presence of a hydrophilic chain transfer agent, N-acetyl-1-cysteine. Batch polymerization conditions were used in these experiments, which were carried out at 80°C for 4 h with 0-3.0 mol% of chain transfer agent. The authors claim that relatively high chain transfer constants were obtained for the complexed monomers, allowing for the degree of polymerization to be controlled efficiently.87,93,94 In spite of these claims, no definitive evidence is provided that would suggest precise aqueous mediated polymerization control with a chain transfer agent.
Modification of cyclodextrins with poly(ethylene oxide) (PEO) is another area of research in the field of polymer synthesis. Topchieva et al. polymerized ethylene oxide off of the primary and secondary hydroxyl groups on β-cyclodextrin.\textsuperscript{95,96} Polymerization of ethylene oxide off of a cyclodextrin core was found to decrease effective cavity size and $K_{\text{assoc}}$. However, there is interest in the amphiphilic properties of this polymer system (Figure 2.9).\textsuperscript{95,96}

![Figure 2.9](http://example.com/image)

Figure 2.9. Structure of poly(ethylene oxide)-cyclodextrin derivative. From: Topchieva, I. N., Mischnick, P., Kuhn, G., Polyakov, V., Elezkaya, S., Bytryzky, G., Karezin, K. Bioconjugate Chem. 1998, 9, 676-682.

A one-pot synthesis was used to polymerize cyclodextrin-initiated ethylene oxide. β-cyclodextrin was dissolved in a 0.9 % NaCl solution (pH 11) in a pressure reactor. EO was added to the reactor, and the reaction was performed at 80°C for 10 h. After purification, DSC characterization of the PEO-cyclodextrin conjugate revealed that the product was amorphous with a $T_g$ of $\sim -70^\circ\text{C}$ (Figure 2.10).\textsuperscript{95,96} NMR and mass spectroscopy characterization revealed that the PEO chains were configured in a random distribution off of the hydroxyls on cyclodextrin. Complexation of these PEO-cyclodextrin conjugates with guest molecules also showed a drastic decrease in $K_{\text{assoc}}$ of their natural cyclodextrin counterparts.\textsuperscript{95,96} The authors claim that this system has future potential as a drug delivery system; however, a more well-defined system is likely needed.
α-Cyclodextrin was used to suppress the phase separation of poly(caprolactone)-b-poly(L-lactic acid) copolymers (PCL-b-PLLA).\textsuperscript{40,97,98} Both of these polymers have different properties, where PLLA has a high tensile strength but is brittle, and PCL has a high flexibility but a low tensile strength. PCL is known to degrade faster than PLLA in biological conditions. Researchers made cyclodextrin inclusion complexes with the PCL-b-PLLA copolymer. The guest polymer chains coalesced together by washing the inclusion complex crystals with water containing a cyclodextrin-degrading enzyme. Different properties were obtained from films formed of the coalesced copolymer and the as-synthesized block copolymer. The coalesced film was determined to have a lower crystallinity using DSC and FTIR (noncrystalline and crystalline absorption bands) characterization, resulting in a faster biodegradation rate (Table 2.4).\textsuperscript{98} Hydrolysis occurs first in the amorphous regions of a semicrystalline polymer. This study shows how the biodegradation rate of PCL-b-PLLA films can be modified using cyclodextrin.
Table 2.4. Thermal properties of homopolymers, as-synthesized block copolymer, and coalesced copolymer. Adapted from: Shuai, X.; Wei, M.; Porbeni, F. E.; Bullions, T. A.; Tonelli, A. E. Biomacromolecules 2002, 3, 201-207.

<table>
<thead>
<tr>
<th>Identity</th>
<th>$T_{m\text{-PCL}}$ (°C)</th>
<th>$\Delta H_{PCL}$ (J/g$_{\text{sample}}$)</th>
<th>$X_{c\text{-PCL}}$ (%)</th>
<th>$T_{m\text{-PLLA}}$ (°C)</th>
<th>$\Delta H_{PLLA}$ (J/g$_{\text{sample}}$)</th>
<th>$X_{c\text{-PLLA}}$ (%)</th>
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</thead>
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<tr>
<td>PCL</td>
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<td>95.6</td>
<td>68.8</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLLA</td>
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<td>-</td>
<td>-</td>
<td>160.5</td>
<td>59.1</td>
<td>63.5</td>
</tr>
<tr>
<td>PCL/PLLA blend</td>
<td>62.1</td>
<td>57.3</td>
<td>64.4</td>
<td>162.4</td>
<td>23.4</td>
<td>70</td>
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<tr>
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<td>42.2</td>
<td>47.4</td>
<td>160.2</td>
<td>22.5</td>
<td>67.2</td>
</tr>
<tr>
<td>Coalesced Copolymer</td>
<td>63.4</td>
<td>22.5</td>
<td>25.3</td>
<td>164.1</td>
<td>5.9</td>
<td>17.6</td>
</tr>
</tbody>
</table>

2.4 Synthesis of Polymeric Cyclodextrin Delivery Vehicles

A major research interest in literature is to synthesize cationic polymers suitable for DNA delivery to cells.\textsuperscript{88,99,100} Examples of traditional cationic polymers that have been investigated for gene delivery are polyethyleneimine and poly(\(\eta\))-lysine. When bound to DNA, polycations condense to nanoparticles often referred to as polyplexes. Polyplexes that contain a net positive charge have the capability of binding to a cell surface. Disadvantageous properties include toxicity and aggregation in physiological conditions. The steric stabilization of polyplexes with PEO via grafting is an established approach to prevent aggregation. However, this method of steric stabilization reduces the charge density of the polymers, lowering DNA binding constants.\textsuperscript{99,100}

Researchers at the California Institute of Technology have published extensively on the development of new types of \(\beta\)-cyclodextrin-containing polycations as nonviral gene delivery vehicles.\textsuperscript{99,100} These polymeric systems are well-defined and show low \textit{in vivo} toxicity with encouraging results in preliminary biological studies. Also, novel methods have been developed
to sterically stabilize these polyplexes without reducing charge density. Formation of inclusion complexes between the cyclodextrin backbone and an adamantane-PEO conjugate allows for stabilization of polyplexes.99

Gonzalez, Hwang, and Davis describe the synthesis of linear β-cyclodextrin-containing polycations.100 Difunctionalized β-cyclodextrin (AA) was copolymerized with a difunctional comonomer (BB), forming the polycation. Diamino-β-cyclodextrin and di(2-aminoethanethio)-β-cyclodextrin monomers were copolymerized with dimethylsuberimidate•2HCl (DMS) or dithiobis(succinimidyl propionate) (DSP) monomers in DMF at 25°C for 15 h. The β-cyclodextrin containing polymer was purified through dialysis (3500 g mol\(^{-1}\) MWCO) against deionized water for 24 h. Scheme 2.2 shows the synthesis of the linear β-cyclodextrin-containing polycations.100 According to the report, the weight average molecular weight of a copolymer synthesized from di(2-aminoethanethio)-β-cyclodextrin and DMS was determined to be 8,800 g mol\(^{-1}\) with a PDI of 1.10. A low yield (24%) was reported for this copolymerization, but the authors claim that this is consistent with other similar copolymerizations. This specific polymer condensed DNA, had a low toxicity profile, and showed in vivo transfections comparable to polyethyleneimine.
Pun and Davis report the synthesis of a similar polymer used to form DNA polyplexes.\textsuperscript{101,102} It was found that the unmodified polyplexes rapidly aggregate in salt solutions, eventually leading to precipitation. To solve this problem, the polyplexes were surface modified by AD-PEO conjugates. These conjugates were synthesized via the reaction of mPEG-succinimidyl propionate with 1-adamantanemethylamine in dichloromethane (25°C) for 12 h. The inclusion complex formed between an AD-PEO and a \(\beta\)-cyclodextrin-containing polyplex was determined to be robust. These PEO-modified polyplexes remained stable in physiological salt conditions, possessing high charge densities and low toxicities. Further studies were performed where targeting ligands, such as galactose and transferrin, were added to the AD-PEO
conjugates for the purpose of specific receptor targeting of the β-cyclodextrin-containing polyplexes.\textsuperscript{99}

Novel diadamantane- and tetraadamantane-PEO conjugates were synthesized for the purpose of forming an extended network of β-cyclodextrin polymers.\textsuperscript{99} This crosslink network was synthesized via the inclusion complexation of the cyclodextrin backbone with the multifunctional adamantane polymers (\textbf{Figure 2.11}).\textsuperscript{99} A linear cyclodextrin-PEO was synthesized via the reaction of diamine-functional β-cyclodextrin and di-Succinimidyl Propionate-PEO in DMSO. This copolymerization gave high polymer yields (~90\%) and PDIs ∼ 1.5. Inclusion complex formation between the multifunctional polymers (1:1 adamantane:cyclodextrin) was performed in water. Network properties were adjusted by changing polymer molecular weights and number of adamantane groups on the guest polymer.


Other groups have studied β-cyclodextrin-containing polycations containing quaternary amine groups. Li et al. synthesized the water-soluble polycations via the reaction of β-cyclodextrin, epichlorhydrin, and choline chloride.\textsuperscript{103} A one-step condensation polymerization
process was carried out in basic aqueous conditions at 60°C. A range of cationic \( \beta \)-cyclodextrin polymers was synthesized. The molar ratio of epichlorohydrin and choline chloride to \( \beta \)-cyclodextrin was adjusted to control the degree of polymerization. The polymers were water soluble, implying that little to no crosslinking occurred during synthesis. Little information was provided about the overall structure of the polymers recovered. The polymers were used to make inclusion complexes with naproxen, a non-steroidal anti-inflammatory drug. Inclusion complex formation with the drug was confirmed using NMR. By altering the molecular weight and the charge density of the polymer, it was found that better drug complexing ability and water solubility than natural \( \beta \)-cyclodextrin could be obtained.

Tang et al. used polyethyleneimines in conjunction with \( \beta \)-cyclodextrin to form a non-toxic nonviral gene delivery vehicle with the capability of drug complexation.\textsuperscript{104} The hydroxyl groups on \( \beta \)-cyclodextrin were reacted with an excess of 1,1’-carbonyldiimidaole in DMF. The degree of substitution and position of the substituents were omitted from the paper. The imidazole-functional \( \beta \)-cyclodextrin was reacted with amine groups on low molecular weight polyethyleneimines (600 g mol\(^{-1}\)). NMR confirmed the appearance of both \( \beta \)-cyclodextrin and polyethyleneimine in the final product. It was determined that 2/3 of the hydroxyl groups on the cyclodextrin were modified with polyethyleneimine. The resulting polyethyleneimine-cyclodextrin polymer was water-soluble and showed a lower toxicity than a 25,000 g mol\(^{-1}\) polyethyleneimine. Gene transfection assay results showed that the cyclodextrin polymer had a gene delivery efficiency comparable to high molecular weight polyethyleneimine homopolymers.

In other research, novel carriers for small interference RNA (siRNA) have been developed. Delivery of siRNA into cells provides selective inhibition of gene expression.\textsuperscript{105,106} Hydrophilic character and high molecular weight inhibits the ability of siRNA to cross biological
membranes. \(\alpha\)-Cyclodextrin conjugates of a generation 3 polyamidoamine dendrimer were synthesized as carriers for siRNA.\(^{106}\) Mono-tosylated \(\alpha\)-cyclodextrin was reacted with the dendrimer in DMSO for 24 h at 60°C. The researchers formed inclusion complexes between siRNA and the starburst polyamidoamine-cyclodextrin dendrimer. The resulting complex displayed negligible cytotoxicity and provided better siRNA delivery to cells than commercially available transfection materials.

Liu, Fan, Kang, and Sun developed a cyclodextrin microgel vehicle for the controlled inclusion complex driven release of drugs.\(^{107}\) \(K_{\text{assoc}}\) is affected by external stimuli, such as temperature and pH. For example, at low pH values, \(K_{\text{assoc}}\) between a cyclodextrin and a drug decreases. This phenomenon was used to design a cyclodextrin-polymer system that would exhibit controlled drug release based on these parameters. A \(\beta\)-cyclodextrin microgel was synthesized via an inverse-emulsion polymerization. \(\beta\)-cyclodextrin and poly(vinyl alcohol) were dissolved in an aqueous sodium hydroxide solution. Epichlorohydrin and emulsifiers (Twen 20/Span 80) were added to the rapidly stirring solution at 60°C for 20 h. After purification, the \(\beta\)-cyclodextrin-microgels were complexed with methyl orange, a model drug. Drug release profiles were performed at a pH of 1.4 and 7.4. As expected, the concentration of free drug in the low pH systems was higher (Figure 2.12).\(^{54}\) This exploitation of pH-dependent \(K_{\text{assoc}}\) for drug release shows how cyclodextrins can be used to develop controlled-delivery systems.
Biodegradable poly(DL-lactide) (PLA)-β-cyclodextrin nanoparticles for drug delivery were prepared by Wang and Ma.\textsuperscript{108} First, a well-defined PLA-cyclodextrin was synthesized. Mono-6-tosyl-β-cyclodextrin was reacted with ethylene diamine to produce mono-6-(2-aminoethyl)amino-β-cyclodextrin. The mono-amine functional cyclodextrin was coupled to PLA using dicyclohexylcarbodiimide in DMF (Scheme 2.3).\textsuperscript{108}

A nanoprecipitation technique was used to prepare PLA-cyclodextrin nanoparticles. A solution of the polymer in acetone (10 mg/mL) was added dropwise to water, and the nanoparticles were recovered using centrifugation. The authors claim that NMR confirmed a nanoparticle structure comprised of a hydrophobic PLA core and a hydrophilic cyclodextrin corona (Figure 2.13).\textsuperscript{108} Studies showed that the PLA-cyclodextrin nanoparticles biodegraded at a much faster rate than
PLA homopolymer nanospheres. It was postulated that the hydrophilic corona allowed for faster water diffusion into the copolymer matrix, increasing degradation rate. Future work with this model system entails the incorporation of drugs and the characterization of release rates.

![Image of PLA-cyclodextrin nanospheres](image)


### 2.5 Addition of Cyclodextrin to Heterobifunctional Poly(ethylene oxide)

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#### 2.5.1 Overview

In this section, the controlled addition of 2-hydroxypropyl-β-cyclodextrin to poly(ethylene oxide) (PEO) is described. In one approach, the hydroxyl terminus of a trivinylsilyl-PEO-OH was modified to contain amine functionality. The trivinylsilyl-PEO-NH₂ was reacted with a 2-hydroxypropyl-β-cyclodextrin derivative containing imidazole functionality, resulting in a
trivinylsilyl-PEO-cyclodextrin. In another approach, 2-hydroxypropyl-β-cyclodextrin was modified to contain an average of one thiol group per ring. This thiol group was added across the electron deficient double bond of a maleimide-PEO-OH, yielding cyclodextrin-PEO-OH.

2.5.2 Experimental

2.5.2.1 Materials

Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. Dimethylsulfoxide (DMSO, 99+%)) and N,N-dimethylformamide (DMF, 99.8%) were purchased from EMD Chemicals and used as received. Dichloromethane (Fischer Scientific, HPLC grade) and diethyl ether (Fischer Scientific, Reagent grade) was used as received. Toluene (Burdick and Jackson, 99.9%) was used as received. 1,1’-Carbonyldiimidazole (97+%), ethylenediamine (99.5+%)) and 2-hydroxypropyl-β-cyclodextrin (0.60 degree of hydroxypropyl substitution) were purchased from Aldrich and used as received. Mercaptoethylamine hydrochloride (98+%)) and triethylamine (99+%)) were purchased from Alfa Aesar and used as received. A double-metal catalyst, Impact™ (Zn₃[Co(CN)₆]₂, Bayer) was diluted with THF to a concentration of 1 mg mL⁻¹. Ethylene oxide (EO, 99.5+%), potassium (98%), hexamethylphosphoramide (HMPA, 99%), sodium iodide (>98%) and 1.6 M vinyl magnesium chloride were purchased from Aldrich and used as received. 3-Chloropropyltrichlorosilane (Gelest) was used as received. Naphthalene (Aldrich, 99%) was sublimed prior to use. A 2.0 M solution of glacial acetic acid (Aldrich) in THF was prepared. Dialysis bags (3,500 g mol⁻¹ MWCO) were obtained from Spectra/Por.

2.5.2.2 Modification of 2-hydroxypropyl-β-cyclodextrin with carbonyldiimidazole

A representative procedure for the addition of an average of one imidazole group on per 2-hydroxypropyl-β-cyclodextrin ring is provided. 2-hydroxypropyl-β-cyclodextrin (2.55 g, 1.85
mmol) and carbonyldiimidazole (0.37 g, 2.31 mmol) were added to a flame-dried, 250-mL roundbottom flask containing a magnetic stir bar and dissolved in DMSO (60 mL). Triethylamine (0.5 mL) was added to the solution and the flask was purged with nitrogen. The reaction was allowed to proceed at room temperature for 2 h. DMSO was removed from the reaction mixture using vacuum distillation at 80°C. The product was washed three times with THF (100 mL). The 2-hydroxypropyl-β-cyclodextrin-imidiazole was dried under vacuum overnight at 60°C and was recovered as a solid white powder. ¹H NMR confirmed the expected chemical structure.

2.5.2.3 Synthesis of thiol containing derivative of 2-hydroxypropyl-β-cyclodextrin

2-Hydroxypropyl-β-cyclodextrin containing an average of one thiol group per ring was prepared using the following procedure. Mercaptoethylamine hydrochloride (0.048 g, 0.42 mmol) was dissolved in DMSO (2 mL). Triethylamine (0.060 mL, 0.42 mmol) was added to the solution, forming insoluble triethylammonium chloride salt. The salt was removed by filtration and the mercaptoethylamine solution was added to a solution of 2-hydroxypropyl-β-cyclodextrin-imidiazole and DMSO (2 mL) in a 50-mL roundbottom flask equipped with a magnetic stir bar. The reaction mixture was stirred at room temperature for 2 h. DMSO was removed from the product using vacuum distillation at 80°C. The product was dissolved in a minimal amount of water (~ 0.5 mL) and was precipitated into THF (40 mL). 2-Hydroxypropyl-β-cyclodextrin containing thiol functionality was recovered using vacuum filtration and dried under vacuum overnight at 40°C. ¹H NMR confirmed the expected chemical structure.

2.5.2.4 Synthesis of trivinylsilyl-functional PEO-OH

The synthesis of a 5,000 g mol⁻¹ trivinylsilyl-functional PEO-OH using a 3-hydroxypropyltrivinylsilane (3-HPTVS) initiator is provided. 3-HPTVS was prepared according to a procedure developed by Vadala et al.³⁵ A 300-mL high-pressure Series 4561 Parr reactor was utilized for the polymerizations. EO (15.2 g, 0.345 mol) was distilled from a lecture bottle
into the pressure reactor cooled with an isopropanol-dry ice bath. THF (10 mL) was added to the reactor via syringe. An initiator solution consisting of 3-HPTVS (0.511 mL, 3.04 mmol), THF (5 mL) and potassium naphthalide (2.9 mL of a 0.95 M solution in THF, 2.7 mmol) was prepared in a separate flame-dried, 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (1.52 mL of a 2.0 M solution in THF, 3.04 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N₂ for 1 h, then opened and its contents were transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The product was washed twice with deionized (DI) water (2X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

2.5.2.5 Functionalization of trivinylsilyl-PEO-OH with carbonyldiimidazole

Trivinylsilyl-PEO-imidizole was synthesized via the reaction of carbonyldiimidazole with the hydroxyl terminus of a 5,600 g mol⁻¹ trivinylsilyl-PEO-OH. Trivinylsilyl-PEO-OH (0.50 g, 0.10 mmol) was charged to a flame-dried, 100-mL roundbottom flask containing a magnetic stir bar and dissolved in THF (6 mL). An excess of carbonyldiimidazole (0.080 g, 0.50 mmol) was added to the flask and dissolved in the solution. The flask was purged with nitrogen and the reaction mixture was stirred at room temperature for 12 h. The product was precipitated into cold diethyl ether (150 mL) and stirred for 30 minutes. Trivinylsilyl-PEO-imidazole was recovered using vacuum filtration and was dried under vacuum overnight at 40°C. H NMR confirmed the expected chemical structure.

2.5.2.6 Reaction of trivinylsilyl-PEO-imidazole with ethylenediamine

Trivinylsilyl-PEO-NH₂ was obtained via the reaction of ethylenediamine with the
activated urethane linkage in a trivinylsilyl-PEO-imidazole. Ethylenediamine (0.025 g, 0.41 mmol) was dissolved in dichloromethane (1 mL) in a 50-mL roundbottom flask equipped with a stir bar. In a separate roundbottom flask, trivinylsilyl-PEO-imidazole (0.42 g, 0.0824 mmol) was dissolved in dichloromethane (5 mL). The polyether solution was added dropwise to the stirring ethylenediamine solution. The reaction mixture was stirred overnight at room temperature. Dichloromethane (200 mL) was added, and the mixture was washed three times with DI water (100 mL). The product was concentrated under vacuum, precipitated in cold diethyl ether (150 mL), and collected using vacuum filtration. The recovered trivinylsilyl-PEO-NH₂ was dried overnight under vacuum at 40°C and characterized using ¹H NMR.

2.5.2.7 Addition of 2-hydroxypropyl-β-cyclodextrin-imidazole to trivinylsilyl-PEO-NH₂

A representative procedure for formation of trivinylsilyl-PEO-cyclodextrin is provided. 5,600 g mol⁻¹ trivinylsilyl-PEO-NH₂ (0.074 g, 0.014 mmol) and 2-hydroxypropyl-β-cyclodextrin-imidazole (0.032 g, 0.022 mmol) were charged to a flame-dried, 100-mL roundbottom flask equipped with a stir bar and dissolved in DMSO (1.5 mL). The flask was purged with nitrogen, and the reaction was allowed to proceed at room temperature for 24 h. The reaction mixture was diluted with DI water (20 mL) and transferred to a 3,500 g mol⁻¹ MWCO dialysis bag. Dialysis was performed against DI water (1 L) for three days to remove DMSO and unbound cyclodextrin. The contents of the dialysis bag were placed in a 100-mL roundbottom flask and lyophilized overnight to recover the product. ¹H NMR was used to characterize the trivinylsilyl-PEO-cyclodextrin.

2.5.2.8 Synthesis of heterobifunctional-poly(ethylene oxide) with a maleimide group on one end (maleimide-PEO-OH)

A maleimide-PEO-OH homopolymer with a molecular weight of 8,600 g mol⁻¹ was prepared via the coordination ring-opening polymerization of EO using a N-(2-hydroxyethyl)maleimide initiator. The synthesis of N-(2-hydroxyethyl)maleimide was previously
described by Thompson et al.\textsuperscript{2} EO (15.4 g) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath. THF (40 mL) was added to the reactor via syringe. An initiator solution consisting of N-(2-hydroxyethyl)maleimide (0.36 g, 2.6 mmol), Zn\textsubscript{3}[Co(CN)\textsubscript{6}]\textsubscript{2} coordination catalyst (Impact\textsuperscript{TM} from Bayer) (0.92 mL of a 1.0 mg mL\textsuperscript{-1} dispersion in THF) and THF (5 mL) 0.91 mL of a 0.94 M solution, 0.85 mmol) was prepared in a separate flame-dried, 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. Additional THF (5 mL) was added to the reaction mixture to ensure quantitative addition of the initiator solution. The cooling bath was removed and the pressure reactor was heated to 90 °C. Polymerization was allowed to proceed until a decrease in pressure was observed (~2.5 h). The reactor was allowed to cool to room temperature and purged with nitrogen for one hour. The reactor was opened and its contents were transferred to a 250-mL, roundbottom flask. The product was concentrated under vacuum, redissolved in dichloromethane (~20 mL) and precipitated into cold diethyl ether (~200 mL). The recovered maleimide-PEO-OH was dried under vacuum at room temperature for 12 h. \textsuperscript{1}H NMR confirmed the expected chemical structure.

2.5.2.9 Reaction of maleimide-PEO-OH with thiol-containing 2-hydroxypropyl-β-cyclodextrin

The double bond of the maleimide group in the polyether was reacted with the thiol-containing 2-hydroxypropyl-β-cyclodextrin to form a thioether linkage. In a representative procedure, a 8,600 g mol\textsuperscript{-1} maleimide-PEO-OH (0.17 g, 0.020 mmol) was charged to a 100-mL roundbottom flask equipped with a magnetic stir bar and dissolved in minimal amount of DMF (~3 mL). 2-Hydroxypropyl-β-cyclodextrin-SH (0.035 g, 0.020 mmol) was added to the solution. The reaction mixture was stirred for 24 h at room temperature. The product was precipitated in cold diethylether (100 mL), recovered using vacuum filtration and dried under vacuum overnight at 40\textdegree{}C. \textsuperscript{1}H NMR was used to characterize the cyclodextrin-PEO-OH.
2.5.2.10 Characterization

Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

2.5.3 Results and Discussion

2-Hydroxypropyl-β-cyclodextrin with a statistical average of one imidazole per ring was synthesized using carbonyldiimidazole (Figure 2.14). The mono-modification derivative of cyclodextrin contained an activated urethane linkage to the imidazole, allowing for further modification with amine-containing compounds. This linkage was stable under the conditions used during the synthesis and workup. Dilute reaction conditions (~5 wt% solids) were employed to prevent the carbonyldiimidazole reagent from reacting with multiple hydroxyl groups on a single 2-hydroxypropyl-β-cyclodextrin ring. The 2-hydroxypropyl-β-cyclodextrin-imidazole product was washed repeatedly with THF to remove excess carbonyldiimidazole and imidazole byproduct.
Figure 2.14. Synthesis of 2-hydroxypropyl-cyclodextrin-imidazole derivative using carbonyldiimidazole.

Adjusting the molar excess of carbonyldiimidazole in the 2-hydroxypropyl-β-cyclodextrin derivatization changed the average number of imidazole groups added to each cyclodextrin ring (Table 2.5). $^1$H NMR was used to quantify the average number of hydroxyl groups modified with carbonyldiimidazole. It was found that a slight molar excess of carbonyldiimidazole yields a 2-hydroxypropyl-β-cyclodextrin imidazole derivative with an average of one imidazole per cyclodextrin ring.

Table 2.5. Molar ratio of 2-hydroxypropyl-β-cyclodextrin to carbonyldiimidazole affects the average number of imidazole groups added to the cyclodextrin ring.

<table>
<thead>
<tr>
<th>2-HP-β-CD:Carbonyldiimidazole Molar Ratio</th>
<th>Average # of Imidazole Groups Per 2-HP-β-CD Ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 : 1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>1.0 : 1.25</td>
<td>1.0</td>
</tr>
<tr>
<td>1.0 : 2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>
\(^1\)H NMR was used to confirm addition of imidazole functionality to the 2-hydroxypropyl-\(\beta\)-cyclodextrin. The 2-hydroxypropyl-\(\beta\)-cyclodextrin had a degree of hydroxypropyl substitution of 0.60, or \(\sim \)4.2 hydroxypropyl groups per \(\beta\)-cyclodextrin ring. As a result, the integration of the pendant methyl proton peak on the hydroxypropyl substituent (\(\sim \) 1.0 ppm) was defined as 12.6 in the \(^1\)H NMR spectra of the imidazole derivatives of cyclodextrin. Comparing this integration to the imidazole protons allowed for determination of the average number of imidazole groups in the reaction product. **Figure 2.15** shows a representative \(^1\)H NMR spectrum of a 2-hydroxypropyl-\(\beta\)-cyclodextrin derivative containing an average of one imidazole group per ring.

**Figure 2.15.** \(^1\)H NMR of mono-modification imidazole derivative of 2-hydroxypropyl-\(\beta\)-cyclodextrin (0.60 degree of hydroxypropyl substitution) in \(d\)-DMSO. The peak at 1.0 ppm is set to an integration of 12.6 due to an average of 4.2 substituted pendant methyl groups per cyclodextrin ring.
The mono-modification imidazole derivative of 2-hydroxypropyl-β-cyclodextrin was reacted with mercaptoethylamine, forming a β-cyclodextrin derivative containing an average of one thiol group per ring (Figure 2.16). The ammonium functionality of mercaptoethylamine hydrochloride was reacted with triethylamine, yielding mercaptoethylamine. The free amine in the mercaptoethylamine was reacted with the carbonyl in the activated urethane linkage of the 2-hydroxypropyl-β-cyclodextrin imidazole derivative. Imidazole byproduct was removed by precipitating the thiol-containing cyclodextrin derivative in THF.

![Diagram](image)

**Figure 2.16.** Synthesis of mono-modification thiol derivative of 2-hydroxypropyl-β-cyclodextrin using mercaptoethylamine.

Addition of mercaptoethylamine to the imidazole derivative of 2-hydroxypropyl-β-cyclodextrin was confirmed using $^1$H NMR (Figure 2.17). Complete disappearance of the imidazole protons, as well as the appearance of methylene protons in the mercaptoethylamine were observed in the $^1$H NMR spectrum of the thiol-containing cyclodextrin derivative.
Quantitative addition of thiol functionality was confirmed by comparing the integration of the pendant methyl groups protons in the hydroxypropyl substituent (1.0 ppm) to the methylene protons next to the thiol endgroup (~ 2.7 ppm).

Figure 2.17. $^1$H NMR shows the disappearance of imidazole protons on a mono-modified 2-hydroxypropyl-$\beta$-cyclodextrin derivative after addition of mercaptethylamine. $d$-DMSO was used as the solvent for the thiol-containing cyclodextrin derivative.

Vadala et al. previously described the synthesis of a well-defined trivinylsilyl-PEO-OH. 3-HPTVS was used as the initiator in the base catalyzed anionic living polymerization of EO. $^1$H NMR and GPC were used to characterize the targeted 5,000 g mol$^{-1}$ heterobifunctional polyether. NMR analysis revealed a PEO molecular weight of 5,600 g mol$^{-1}$ (Figure 2.18). The number
average molecular weight ($M_n$) of the trivinylsilyl-PEO-OH was determined to be 5,200 g mol$^{-1}$ with a polydispersity index (PDI) of 1.09 by GPC analysis.

**Figure 2.18.** $^1$H NMR of a 5,600 g mol$^{-1}$ trivinylsilyl-PEO-OH.

Trivinylsilyl-PEO-imidazole was prepared by reacting the trivinylsilyl-PEO-OH with carbonyldiimidazole (Figure 2.19). An activated urethane linkage was formed between the hydroxyl terminus of the trivinylsilyl-functional polyether and an imidazole group. The product was precipitated into diethyl ether to remove excess carbonyldiimidazole and imidazole byproduct.
Quantitative addition of imidazole to the hydroxyl terminus of the polyether was confirmed using $^1$H NMR. The integrations of the imidazole protons (7.0 ppm, 7.5 ppm and 8.2 ppm) were compared to the protons of the trivinylsilyl-terminus (~6 ppm), revealing a 1:1 stoichiometric ratio of endgroups. Additionally, a quantitative shift of the methylene protons next to the oxygen of the urethane linkage to ~ 4.5 ppm was observed. Figure 2.20 shows the $^1$H NMR spectrum of the 5,600 g mol⁻¹ trivinylsilyl-PEO-imidazole.
Figure 2.20. $^1$H NMR illustrated the appearance of imidazole protons on a 5,600 g mol$^{-1}$ trivinylsilyl-PEO-imidazole. A 1:1 stoichiometric ratio of endgroups confirms quantitative conversion the hydroxyl terminus.

Ethynediamine was reacted with the trivinylsilyl-PEO-imidazole, yielding a heterobifunctional polyether with an amine terminus (Figure 2.21). A dilute solution of the trivinylsilyl-PEO-imidazole dissolved in dichloromethane was added dropwise to a solution of ethynediamine to prevent coupling of the polyether chains. A urethane linkage was formed between the trivinylsilyl-functional polyether and ethylene diamine. The purified trivinylsilyl-PEO-NH$_2$ was characterized using $^1$H NMR and GPC.
Addition of ethylenediamine to the imidazole-functional endgroup of the trivinylsilyl-PEO-imidazole was confirmed using $^1$H NMR. The imidazole proton peaks completely disappeared and methylene proton peaks attributable to ethylenediamine (2.7 ppm and 3.2 ppm) appeared in the spectrum. Comparison of proton peak integrations of the endgroups showed quantitative addition of ethylene diamine. Figure 2.22 shows the $^1$H NMR spectrum of the trivinylsilyl-PEO-NH$_2$. The amine terminus of the trivinylsilyl-PEO-NH$_2$ was endcapped with phenyl isocyanate prior to GPC characterization. GPC analysis revealed a $M_n$ of 5,400 g mol$^{-1}$ and a PDI of 1.15 for the endcapped amine-functional polyether, indicating no occurrence of polyether chain coupling.
Figure 2.22. $^1$H NMR shows that a 1:1 stoichiometry of endgroups is retained during the synthesis of trivinylsilyl-PEO-NH$_2$.

 Cyclodextrin was attached to the trivinylsilyl-PEO-NH$_2$ via the reaction of the imidazole derivative of 2-hydroxypropyl-β-cyclodextrin with the amine terminus of the polyether (Figure 2.23). This well-defined polymer consists of one β-cyclodextrin ring per polyether chain, bound by two urethane linkages. Unbound cyclodextrin was removed using dialysis. The cyclic oligosaccharide endgroup of the trivinylsilyl-PEO-cyclodextrin contained a large number of functionalizable hydroxyl groups and allowed for inclusion complexation with hydrophobic molecules. Functionalization of the trivinylsilyl-endgroups with a suitable magnetite anchor group would allow for the formation of a novel polyether magnetite stabilizer, yielding controlled cyclodextrin-PEO magnetite complexes.
Figure 2.23. Synthesis of trivinylsilyl-PEO-cyclodextrin.

Figure 2.24 shows the $^1$H NMR spectrum of the trivinylsilyl-PEO-cyclodextrin in $d$-DMSO. Appearance of peaks corresponding to the $\beta$-cyclodextrin ring and the pendant methyl group of the hydroxypropyl substituents were observed. Integration of the endgroup peaks revealed that the cyclodextrin and trivinylsilyl endgroups show approximately a 1:1 stoichiometric ratio.
Thompson et al. previously described the preparation of a heterobifunctional maleimide-PEO-OH using a N-(2-hydroxyethyl)maleimide initiator. A Zn₃[Co(CN)₆]₂ coordination catalyst (Impact™ from Bayer) was utilized for the polymerization of EO to prevent side reactions with the maleimide functional group. ¹H NMR characterization of the maleimide-PEO-OH revealed a $M_n$ of 8,600 g mol⁻¹, and confirmed the presence of the maleimide double bond (~6.7 ppm) (Figure 2.25). A $M_n$ of 7,800 g mol⁻¹ and a PDI of 1.87 for the maleimide-PEO-OH was determined using GPC.
The addition of cyclodextrin to the endgroup of a polyether was also achieved using the reaction between a thiol and an electron-deficient maleimide double bond.\(^2\)\(^{109}\) In this reaction, a thioether linkage was formed between the thiol-containing 2-hydroxypropyl-β-cyclodextrin and the maleimide-PEO-OH (Figure 2.26). The two reactants were added in equal molar amounts and the product was precipitated in diethyl ether. \(^1\)H NMR was used to characterize the cyclodextrin-PEO-OH product. Using this approach, the hydroxyl terminus of the maleimide-PEO-OH could be functionalized and anchored to a magnetite nanoparticle surface. Subsequently, the thiol-containing 2-hydroxypropyl-β-cyclodextrin could be added to the maleimide-PEO magnetite complex, allowing for the formation of a cyclodextrin-PEO magnetite complex.
Figure 2.26. Reaction of thiol-containing 2-hydroxypropyl-β-cyclodextrin with maleimide-PEO-OH.

Figure 2.27 shows the $^1$H NMR spectrum of the cyclodextrin-PEO-OH in $d$-DMSO. The maleimide double bond peak in the polyether precursor disappeared after the reaction with the thiol-containing 2-hydroxypropyl-β-cyclodextrin. Additionally, peaks associated with the cyclodextrin ring and substituents appeared in the spectrum.
2.6 Conclusions

Cyclodextrins are cyclic molecules that have a hydrophilic exterior and a hydrophobic cavity. These molecules possess the unique ability to form inclusion complexes with lipophilic molecules. This host/guest interaction is characterized by the association constant, $K_{assoc}$. Cyclodextrin molecules contain many potential modification sites (primary and secondary hydroxyl groups), from which many derivatives may be synthesized. These derivatives can be used to increase the water solubility of cyclodextrin, increase host/guest interactions, and/or bind to polymers.

Cyclodextrins have been used in polymer synthesis for many applications. Cyclodextrin can increase the aqueous solubility of hydrophobic monomers, such as methacrylates and styrene.
The cyclic molecule can also initiate ethylene oxide monomer from its many hydroxyl groups. Cyclodextrin polymers for use as nonviral gene delivery vectors are also being developed. The unique properties of cyclodextrin will ensure that its disparate uses in the field of polymers will continue to grow in the future.

2-Hydroxypropyl-β-cyclodextrin was added to poly(ethylene oxide) using two approaches. Characterization of these polymers shows an approximate 1:1 stoichiometry of PEO to cyclodextrin. These polymers could be further functionalized to contain anchor groups for the stabilization of magnetite.
CHAPTER 3: Synthesis and Colloidal Properties of Polyether-Magnetite Complexes in Water and Phosphate Buffered Saline

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3.1 Abstract

Biocompatible magnetic nanoparticles show great promise for many biotechnological applications. This paper addresses the synthesis and characterization of magnetite nanoparticles coated with poly(ethylene oxide) (PEO) homopolymers and amphiphilic poly(propylene oxide-b-ethylene oxide) (PPO-b-PEO) copolymers that were anchored through ammonium ions. Predictions and experimental measurements of the colloidal properties of these nanoparticles in water and phosphate buffered saline (PBS) as functions of the polymer block lengths and polymer loading are reported. The complexes were found to exist as primary particles at high polymer compositions and most formed small clusters with equilibrium sizes as the polymer loading was reduced. Through implementation of a polymer brush model, the size distributions from DLS were compared to those from the model. For complexes that did not cluster, the experimental sizes matched the model well. For complexes that clustered, equilibrium diameters were predicted accurately through an empirical fit derived from DLS data and the half-life for doublet formation calculated using the modified Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. Deviation from this empirical fit provided insight into possible additional interparticle hydrophobic interactions for select complexes for which the DLVO theory could not account. While the
polymers remained bound to the nanoparticles in water, most of them desorbed slowly in PBS. Desorption was slowed significantly at high polymer chain densities and with hydrophobic PPO anchor blocks. By tailoring the PPO block length and the number of polymer chains on the surface, flocculation of the magnetite complexes in PBS was avoided. This allows for in vitro experiments where appreciable flocculation or sedimentation will not take place within the specified timescale requirements of an experiment.

3.2 Introduction

The properties of magnetite nanoparticles make them ideal for biomedical applications including cell separations, treatment of detached retinas and use as contrast-enhancing agents for MRI. Their low cytotoxicities make them promising candidates for in vivo applications. In addition, the magnetite surface is amenable to adsorption of biocompatible macromolecules. Thus, dispersants and targeting or imaging agents can be bound to these nanoparticles.

At room temperature, magnetite nanoparticles below approximately 23 nm in diameter exhibit superparamagnetic behavior, and their colloidal properties can be influenced by interparticle magnetic attractive forces when they are placed in an external magnetic field. When a magnetic field is applied, the magnetic dipoles align in the field direction and the particles begin to self-assemble in the absence of repulsive interactions. Attaching soluble nonionic polymer chains to form a brush on a surface generally increases colloidal stability and inhibits aggregation due to steric repulsion. This arises from a combination of enthalpic and entropic effects resulting from the confinement of soluble tail chains in the gap between two particles. Steric stabilization occurs when the steric forces keep the particles separated by a
distance that prevents aggregation due to attractive forces. With sufficiently thick brushes, the interaction potentials can be entirely repulsive, resulting in a very stable dispersion.\textsuperscript{126} In addition, by choosing the correct stabilizer molecular weight and chain density on the magnetite surface, we show herein that small controlled clusters of these complexes can form, while still maintaining sufficient steric forces to disperse the clusters. This can occur even in the absence of an applied field.

Various steric stabilizers for magnetite have included polyvinylpyrrolidone, dextran, polydimethylsiloxane and PEO.\textsuperscript{4,5,111,119,120,127} Adsorption of these polymers onto the surface of magnetite is enhanced by introducing specific functional anchor groups.\textsuperscript{4,5,7,111} In aqueous media, amphiphilic block copolymers with controlled hydrophobic and hydrophilic blocks can also contribute to polymer adsorption and better colloidal stability as reported herein. Previous work in our laboratories has utilized poly(propylene oxide-\textit{b}-ethylene oxide) (PPO-\textit{b}-PEO) block copolymers functionalized with carboxylates on the hydrophobic end to adsorb onto the magnetite surface, but the lack of stability of those complexes in physiological media is a concern.\textsuperscript{3-5,7,111}

This paper describes magnetite nanoparticles that have been sterically stabilized with homo- and diblock copolyethers having three alkylammonium groups on one end to promote adsorption onto the magnetite surface. The three main thrusts include 1) relationships of stabilizer chain densities and hydrophobic interactions to colloidal stabilities of the complexes, 2) influences of material parameters such as polymer composition and loading on forming controlled clusters of the complexes, and 3) the suitability of alkylammonium ions as anchor groups for the magnetite nanoparticles in physiological media, particularly in solutions that contain phosphate salts. Additionally, by observing deviations of experimental data from the model, aggregation phenomena are observed that would otherwise go unseen. We believe that developing an
understanding of these three areas will enable the design of magnetic complexes wherein sizes, clustering, and interactions with physiological media and with cells can be controlled through judicious choices of molecular parameters.

3.3 Experimental

3.3.1 Materials

Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), ethylene oxide (EO, 99.5±%), cysteamine hydrochloride, potassium (98%), hexanes (HPLC grade), hexamethylphosphoramide (HMPA, 99%), propylene oxide (PO, >99%), oleic acid, NaOH (1 N, aq), sodium iodide (>98%), iron(III) acetylacetonate (Fe(acac) ) and 1.6 M vinyl magnesium chloride were purchased from Aldrich and used as received. 3-Chloropropyltrichlorosilane (Gelest) was used as received. Dichloromethane and nitric acid (0.1 N) (Fisher Scientific) were used as received. Naphthalene (Aldrich, 99%) was sublimed prior to use. Glacial acetic acid (Aldrich) was diluted with THF to yield a 2.5 M solution. N,N-Dimethylformamide (DMF, EMD Chemicals) was dried over CaH₂, fractionally distilled under vacuum and stored under nitrogen at 25 °C. A double-metal catalyst, Impact™, was kindly donated by Bayer and was diluted with distilled THF to yield a 4.1 mg mL⁻¹ dispersion. Dulbecco’s PBS (10X, Aldrich) without calcium and magnesium was diluted to a concentration of 1X.
3.3.2 Synthesis of trivinylsilyl-functional PEO

A characteristic procedure for the synthesis of a trivinylsilyl-initiated PEO is provided. A 7.2k $M_n$ PEO was initiated with 3-hydroxypropyltrivinylsiline (3-HPTVS). 3-HPTVS was prepared according to a procedure developed by Vadala et al.\textsuperscript{2,3} A 300-mL high-pressure Series 4561 Parr reactor was utilized for the polymerizations. EO (20.4 g, 0.463 mol) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath. THF (40 mL) was added to the reactor via syringe. An initiator solution consisting of 3-HPTVS (0.704 mL, 6.80 mmol), THF (10 mL) and potassium naphthalene (0.91 mL of a 0.94 M solution, 0.85 mmol) was prepared in a separate flame-dried, 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (2.72 mL of a 2.0 M solution in THF, 6.80 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N\textsubscript{2} for 1 h, then opened and its contents were transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The product was washed twice with deionized (DI) water (2X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

3.3.3 Synthesis of trivinylsilyl-functional PPO-b-PEO (trivinylsilyl-PPO-b-PEO-OH)

A representative procedure for a 5.6k PPO-\textit{b}-7.2k PEO ($M_n$) trivinylsilyl-functional copolymer is provided. PO (10 g, 0.175 mol) was added to a 300-mL pressure reactor via syringe, followed by 5 mL of distilled THF. An initiator solution consisting of 3-HPTVS (0.3 g, 1.8 mmol), THF (5 mL), and Impact\textsuperscript{TM} catalyst (0.25 mL, 100 ppm based on PO) was prepared in
a flame-dried, 100-mL roundbottom flask. The initiator dispersion was agitated rapidly for 10 min, then introduced into the pressure reactor, and the reactor was charged with N₂ to a pressure of 30 psi. The polymerization was conducted at 90 °C until a cessation in pressure drop was observed (~3 h). After cooling to room temperature and purging with nitrogen, the reactor was opened and the reaction mixture was transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum, yielding trivinylsilyl-functional PPO.

EO (20.4 g, 0.463 mol) was distilled from a lecture bottle into a 300-mL pressure reactor cooled with an isopropanol-dry ice bath. THF (10 mL) was added to the reactor via syringe. In a 50-mL roundbottom flask, the hydroxy terminus of the trivinylsilyl-functional PPO (5 g, 0.9 mmol) was reacted with potassium naphthalene (0.91 mL of a 0.94 M solution, 0.85 mmol) in THF (5 mL) to form a PPO alkoxide macroinitiator. The macroinitiator solution was added to the pressure reactor via a syringe. The polymerization and polymer isolation conditions were identical to those for the PEO homopolymer described above.

3.3.4 Functionalization of trivinylsilyl-functional polyethers with amines

Polyethers with three amine groups on one terminus were obtained via the ene-thiol addition of cysteamine hydrochloride across the vinylsilane groups. In a characteristic procedure, trivinylsilyl-PPO-\textit{b}-PEO-OH (2 g, 0.16 mmol), cysteamine hydrochloride (80 mg, 0.7 mmol), and AIBN (26 mg, 0.15 mmol) were dissolved in deoxygenated DMF (6 mL) in a roundbottom flask. The reaction was conducted at 80 °C for 24 h with stirring, then the reaction mixture was cooled to room temperature. DI water (100 mL) was added to the flask, and the mixture was transferred to a separatory funnel. Dichloromethane (200 mL) was added to the separatory funnel to extract the alkylammonium-functionalized polyether from the water layer. The dichloromethane layer was washed with a 1 N solution of sodium bicarbonate (3X), followed by three washes with DI
water. The dichloromethane was removed under vacuum, and the triamine-PPO-\textit{b}-PEO-OH copolymer was dried at 50 °C under vacuum for 12 h. $^1$H NMR confirmed the expected chemical structure.

3.3.5 Magnetite synthesis via reduction of Fe(acac)$_3$

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al.$^8$ Fe(III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were added to a 250-mL, three-necked roundbottom flask equipped with a water condenser and overhead mechanical stirrer. N$_2$ was passed through the solution for 1 h. While stirring under N$_2$, the solution was heated to 100 °C for 4 h, then the temperature was increased to 205 °C at a rate of ~25 °C h$^{-1}$. Following 24 h at 205°C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone, then were dispersed in chloroform (20 mL) containing oleic acid (0.3 g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone. The particles were dried under vacuum for 24 h at 25°C. The composition of the particles obtained from thermogravimetric analysis (TGA) showed 5% organic residue to 95% magnetite.

3.3.6 Adsorption of polyether stabilizers onto magnetite nanoparticles

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50 mg) prepared as described above were dispersed in chloroform (10 mL) and added to a 50-mL roundbottom flask. A triamine-functional polyether (100 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N$_2$, and then the nanoparticles were precipitated in hexanes (300 mL). A magnet
was utilized to collect the magnetite nanoparticles and free oleic acid was decanted with the supernatant. The complexes were dried under N₂ at 25 °C for 24 h. The complexes were then dispersed in aqueous media using a Tekmar TM-200 probe-tip sonicator at medium power for 20-30 s.

3.3.7 Characterization

Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

TGA was carried out on the PEO-stabilized and PPO-b-PEO-stabilized magnetite nanoparticles using a TA Instruments TGA Q500. Each sample was first held at 110 °C for 10 min to drive off any excess moisture. The sample was then equilibrated at 100 °C and the temperature was ramped at 10 °C min⁻¹ to a maximum of 600 °C in a nitrogen atmosphere. Char yields (the mass remaining at a particular temperature) were recorded throughout the experiment.

TEM was conducted with a Philips EM-420 field-emission-gun transmission electron microscope. The microscope was equipped with a 2000 x 3000 pixel digital imaging system. Dry samples of the magnetite complexes were dispersed in water and cast onto amorphous carbon-coated copper grids for analysis. Great care was taken to ensure that both eucentric height and focus were set consistently from one sample to another to reduce uncertainty in the digital image analyses. Images were acquired at a magnification of 96 kx, corresponding to a resolution of 3.7 pixels nm⁻¹. This magnification gave both sufficient resolution and contrast for digital
image analysis, and provided a large enough field of view to obtain adequate numbers of particles. Particle distribution analyses were performed using Reindeer Graphics’ Fovea Pro 4 plug-in for Adobe Photoshop 7.0.

DLS measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Intensity, volume and number average diameters were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters. Zeta potential measurements were also performed with the Zetasizer ZS at 25 ± 0.1°C to measure the particle velocity imparted due to an applied electric field which can be related to the surface charge.

Each complex was dispersed in DI water as described above. The solution was then diluted to approximately 0.05 mg mL⁻¹ and filtered through a Whatman Anotop 100-nm alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of 1.3 × 10⁻⁵ to 2.2 × 10⁻⁵ depending on the polymer loading. In experiments where PBS was added, 0.1 mL 10X PBS was added to 0.9 mL of the 0.05 mg mL⁻¹ complex solution and the new solution was mixed and then filtered through a 100-nm alumina filter into a clean polystyrene cuvette. Each sample was analyzed directly following filtration and measured either 3 times in a 5-min period or every 30 minutes over 24 hours depending on the experiment.

Zeta potential measurements were taken by dispersing the sonicated complexes in either 25 mL of DI water or 1X PBS at a concentration of ~0.05-0.1 mg mL⁻¹. The pH was adjusted up to ~7.5-8.5 using 0.1 N sodium hydroxide. The zeta potential was measured as the pH was decreased in small increments using 0.1 N nitric acid. Both nitric acid and sodium hydroxide
were determined to be indifferent electrolytes and so they did not greatly affect the surface charge density of the magnetite.

Powder X-Ray Diffraction (XRD) patterns were obtained using a Scintag XDS-2000 diffractometer with a Ni-filtered Cu-Kα (λ = 0.154 nm) radiation source. The patterns were obtained at a scan rate of 1.0 2θ s\(^{-1}\) and were scanned from 10 to 90\(^{\circ}\). Particle diameters were obtained using the Scherrer formula, which allows for estimation of particle diameter as a function of the width of the diffraction curves (Eq. 3.1).

\[
d_{XRD} = \frac{0.9\lambda}{\beta \cos \theta}
\]

Eq. 3.1

Here, λ is the wavelength of radiation, β is the peak width at half height in radians, and θ is the angle of reflection.\(^{129}\)

3.3.8 Modeling to Predict Solution Sizes and Colloidal Stabilities of the Complexes

The magnetite particle core radii were determined through analyses of TEM images and by XRD. Figure 3.1 shows an original and the contrast-enhanced TEM image used for image analysis. At least 2000 particles per sample were analyzed and each image was scrutinized manually to correct software errors. The equivalent radii were graphed versus the probability of their occurrence and this histogram was fit with a Weibull probability distribution as shown in Equation 3.2.

\[
P(a) = \frac{c}{b} \left(\frac{a}{b}\right)^{c-1} \exp\left[\left(\frac{a}{b}\right)^c\right]
\]

Eq. 3.2
Here, $c$ is the Weibull scale parameter, $b$ is the Weibull shape parameter, and $a$ is the particle radius.

**Figure 3.1.** Probability histograms of complexes were generated by modifying the original image (A) to a contrast-enhanced image (B) using Fovea Pro 4.0 software. The images for a complex with a triammonium-5.6kPPO-b-7.2kPEO-OH copolymer are shown.

**Figure 3.2** shows two histograms of particle radii versus probability for a triammonium-7.2kPEO-magnetite complex and a triammonium-5.6kPPO-b-7.2kPEO-OH diblock complex. Each complex was prepared with a different batch of magnetite, and the variability between the histograms and their respective probability distributions is well within standard error. The particle radii reported herein are equivalent radii assuming the particles were spherical. XRD was
performed on the particles to confirm the magnetite composition and to determine the average particle diameter using the Scherrer equation, which also assumes a spherical particle. The diameters based on five different XRD peaks were averaged, giving a diameter of $8.2 \pm 1.2$ nm, which compares favorably to the diameters of $7.8 \pm 2.2$ nm (5.6kPPO-b-7.2kPEO-OH) and $8.1 \pm 2.9$ nm (7.2kPEO-OH) obtained from the images in Figure 3.2. Thus, the validity of assuming a spherical particle was supported by the consistency of data derived from the multiple methods.

![Probability histograms for a triammonium-5.6kPPO-b-7.2kPEO-OH complex (gray) and a triammonium-7.2kPEO-OH complex (black) indicate that the magnetite core size distribution is reproducible.](image)

**Figure 3.2.** Probability histograms for a triammonium-5.6kPPO-b-7.2kPEO-OH complex (gray) and a triammonium-7.2kPEO-OH complex (black) indicate that the magnetite core size distribution is reproducible.

Hydrodynamic radii ($R_m$) of the magnetite-polymer complexes were calculated by the method of Mefford, et al.$^9,10$ TEM image analysis data was first fitted with a Weibull probability distribution to calculate an average specific surface area of the magnetite particles. Combining
average surface area with the average polymer loading per mass of the complex (from TGA) leads to an average number of chains per magnetite surface area, $\alpha$, as shown in **Equation 3.3**.

$$\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag}}{MW_{Total} W_{mag}} \int_{0}^{\infty} \left( \frac{3}{a} \right) P(a) da$$

Eq. 3.3

Here, $\rho_{mag}$ is the density of magnetite (5.21 g cm$^{-3}$), $W_{mag}$ is the weight fraction of the complex that is magnetite, and $MW_{Total}$ is the polymer number average molecular weight. Using a modification of Vagberg’s density distribution model, we calculated the number of chains per particle, $f(a)$, and the complex radius $R_{m}(a)$—the radius of the particle plus its attached brush—as

$$f(a) = 4 \pi a^2 \alpha$$

Eq. 3.4

$$R_{m}(a) = \left( \frac{8 N_{k} f(a) L_{k}^{1/\nu} + a^{1/\nu}}{4^{1/\nu} 3^{\nu}} \right)^{\nu}$$

Eq. 3.5

where $N_{k}$ is the number of statistical segments in the PEO chain (63), $\nu$ is the Flory exponent of PEO in water at 25°C (0.583), $L_{k}$ is the length of a statistical segment (0.6 nm), and $a$ is the particle radius. For complexes with the PPO-$b$-PEO diblock copolymers, the PPO layer was assumed to be a non-solvated, hydrophobic shell surrounding the magnetite core and the PEO was modeled as concentric blobs extending out from the surface of the PPO layer. To account for the PPO layer, the model was adapted to include a new effective core size, which is the radius of the magnetite core obtained from the Weibull distribution plus the thickness of the hydrophobic anchor layer ($t_{a}$) (Eq. 3.6).
Here, $\rho_{\text{PPO}}$ is the density of the PPO anchor layer (1.04 g cm$^{-3}$), $\text{MW}_{\text{PPO}}$ is the anchor layer number average molecular weight, and $\text{MW}_{\text{Total}}$ is the total polymer number average molecular weight. Thus, the predicted hydrodynamic radius of the complex as a function of magnetite particle radius can be described by \textbf{Equation 3.7}.

$$R_m(a) = \left( \frac{8N_k f(a)^{1-v}}{4^{1/v} 3^v} L_k^{1/v} + (a + t_a)^{1/v} \right)^v$$ \textbf{Eq. 3.7}

This function along with the Weibull distribution function for the magnetite particle radius is used to determine radial distribution averages for comparison with DLS experimental data. Each average weights particles of various diameters differently and thus gives an estimate of the homogeneity of the particle systems. The number average diameter, $D_n$, of the magnetite complex can be calculated as

$$D_n = 2 \int_{0}^{\infty} R_m(a) P(a) da$$ \textbf{Eq. 3.8}

The volume average diameter, $D_v$, of the magnetite complex can be calculated as
Using a method by Mefford, et al. and assuming the particles are in the Rayleigh scattering regime, the intensity average diameter, $D_i$, for the magnetite complex was calculated as

$$D_i = 2 \frac{\int_0^\infty R_m(a)^6 P(a)da}{\int_0^\infty R_m(a)^5 P(a)da}$$  \text{Eq. 3.10}$$

As Equations 3.8-3.10 indicate, the intensity average diameter weights larger particles significantly more than the volume average diameter, which in turn, weights larger particles more than the number average diameter. Thus, if a system has small amounts of clustered nanoparticles, this would affect the intensity average more than the number average. This is important when considering which weighted average from the model to compare to the DLS results. If the model matches the measurements obtained via DLS closely, the model can then be used with DLVO calculations to predict stabilities of the complexes in solution.

Colloidal stability can be predicted from a model for the total potential energy $V_{\text{Total}}$ as described by DLVO theory and shown in Equation 3.11.$^{126}$

$$V_{\text{Total}} = V_{\text{vdW}} + V_M + V_{\text{ES}} + V_S$$  \text{Eq. 3.11}$$

$V_{\text{vdW}}$ is the attractive potential due to van der Waal’s interactions, $V_M$ is the attractive potential produced by an applied magnetic field, $V_{\text{ES}}$ is the repulsive potential due to electrostatic interactions, and $V_S$ is the repulsive potential due to steric repulsion of the polymer brushes. The
magnetic potential energy term was neglected because for the stability calculations there is no magnetic field being applied to the system. The electrostatic potential can be calculated as

\[ V_{ES} = 2\pi a \varepsilon \varepsilon_o \psi_o^2 \ln \left(1 + e^{-\kappa(r-2a)}\right) \]

Eq. 3.12

where \( \varepsilon \) is the dielectric constant of the solvent, \( \varepsilon_o \) is the permittivity of free space, \( \psi_o \) is the surface potential, \( \kappa \) is the inverse Debye length, \( r \) is the center-to-center separation, and \( a \) is the particle radius.\(^{133} \) The van der Waal’s potential can be calculated as

\[
V_{vdW} = -\frac{1}{6} A_{eff} \left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln \left(\frac{r^2 - 4a^2}{r^2}\right)\right)
\]

Eq. 3.13

\( A_{eff} \) is the effective retarded Hamaker constant calculated from Equation 3.14.

\[
A_{eff} = A_{v=0} + A_{v>0} = \frac{3}{4} kT \left(\varepsilon(0) - \varepsilon(0)\right)^2 + \frac{3\hbar \omega}{16\sqrt{2}} \frac{(n_o^2 - n_o^2)^2}{(n_o^2 + n_o^2)^{3/2}} F(H)
\]

Eq. 3.14

In this equation, \( k \) is Boltzmann’s constant, \( T \) is the temperature, \( \varepsilon(0) \) (8.0 \times 10^1)\(^{134} \) and \( \varepsilon(0)(2.0 \times 10^4) \)\(^{135} \) are the dielectric constants for the medium (water) and the substrate (magnetite), \( n_o(1.33) \)\(^{134} \) and \( n_o(1.97) \)\(^{134} \) are the refractive indices (in the visible range) of the medium and substrate, \( \hbar \) is the reduced Planck’s constant, and \( \omega \) (1.88 \times 10^{16} \text{ rad/sec})\(^{134} \) is the frequency of the dominant relaxation in the UV. \( F(H) \) accounts for retardation effects and is unity at the nonretarded limit.\(^{134} \) For this treatment, the Hamaker constant was calculated for magnetite only, and was 9.0 \times 10^{-20} \text{ J} in the nonretarded limit. The Hamaker constant for magnetite has been reported elsewhere as 16.4 \times 10^{-20} \text{ J}.\(^{136} \) Our calculation neglects the contributions of the anchor layer thickness and adsorbed polymer brush. Typically Hamaker constants for polymers are significantly lower than for metal oxides and so the magnetite contribution should be greater than
the polymer contribution to the overall Hamaker constant. Further refinement of the model will incorporate a core/shell estimation of the Hamaker constant.

For the case of a densely adsorbed brush layer, the steric contribution to the interaction potential can be described by the expression\textsuperscript{137}

\[
V_s = \frac{5}{18} kT f(a)^{\frac{3}{2}} \left\{ \begin{array}{ll}
-\ln \left( \frac{r}{\sigma} + \frac{1}{2f(a)} \sqrt{f(a)} \right); & r \leq \sigma \\
\frac{1}{2f(a)} \left( \frac{\sigma}{r} \right) \exp \left( -\frac{1}{2\sigma} \sqrt{f(a)} (r-\sigma) \right); & r > \sigma 
\end{array} \right. 
\]

\textsuperscript{Eq. 3.15}

Here, \( f(a) \) is the number of chains per particle (\textbf{Equation 3.4}) and \( \sigma/2 \) is the distance from the center of the core to the center of the outermost blob layer.\textsuperscript{133,137} For this expression, \( \sigma \) is defined as \( 1.3R_g \), the radius of gyration of the ensemble.\textsuperscript{133} The inner, logarithmic term in the equation which describes close approaches was derived originally by Witten and Pincus\textsuperscript{138} to describe the entropic repulsion when chains from adjacent star-like objects overlap. The outer, exponential term (\( r>\sigma \)) is based on a Yukawa-type decay (such as that used to describe a screened Coulomb potential\textsuperscript{139}) with the decay length set to the diameter of the largest blobs and has been extensively tested using machine simulations.\textsuperscript{137}

The radius of gyration, \( R_g \), can be approximated using the previously described model and the Weibull particle radius distribution fit by calculating the moment of inertia of the complex as described by Mefford et al.\textsuperscript{9,10} The moment of inertia of a mass, \( m \), rotated about an axis at a distance \( x \) is given by\textsuperscript{140}
\[ I = \chi^2 dm \quad \text{Eq. 3.16} \]

Extension of this to the moment of inertia of each component of the complex can be calculated as

\[ I_{\text{Total}} = I_{\text{Mag}} + I_{\text{PPO}} + I_{\text{PEO}} = \int_0^a \rho_{\text{mag}} 4\pi a^4 da + \int_a^{a+\tau} \rho_{\text{PPO}} 4\pi a^4 da + \int_{a+\tau} R_g(a) \left( \frac{a}{\tau} \right) \frac{1}{\nu} 4\pi ada \quad \text{Eq. 3.17} \]

The total mass of the complex can be calculated as

\[ M_{\text{Total}} = M_{\text{Mag}} + M_{\text{PPO}} + M_{\text{PEO}} = \frac{4}{3} \pi a^3 \rho_{\text{mag}} + \frac{M_{\text{mag}} (1 - W_{\text{mag}})}{W_{\text{mag}}} \quad \text{Eq. 3.18} \]

and the radius of gyration is defined as

\[ R_g = \int \frac{I_{\text{Total}}}{M_{\text{Total}}} P(a) da \quad \text{Eq. 3.19} \]

To test this method, the calculated \( R_g \)s were compared to measured values of \( R_g \) for polymer micelles from small angle neutron scattering (SANS) with agreement within 7% of experimental values.\(^{141}\) This allows for calculation of the stability ratio and the half-life for doublet formation using Equations 3.20 and 3.21, respectively.

\[ W = 2\bar{a} \int_{2\bar{a}}^{\infty} e^{\frac{-r}{kT}} \frac{1}{r^2} dr \quad \text{Eq. 3.20} \]

\[ t_{1/2} = \frac{3}{\bar{a} \pi \mu W} \frac{1}{f \nu} \quad \text{Eq. 3.21} \]

Here, \( \mu \) is the solvent viscosity, \( \Phi \) is the volume fraction of particles in solution, and \( \bar{a} \) is the average particle radius.\(^{126,142}\) The classical expression for \( W \) does not account for polydispersity and only concerns pair interactions for particles of like sizes. We account for the size distribution...
by computing $\bar{a}$ (4.0 nm) from the Weibull distribution (Equation 3.2). The half-life for doublet formation is the time required for half of the particles to flocculate in the form of doublets. This parameter serves as a basis of comparison for complexes with different polymer molecular weights.

### 3.4 Results and Discussion

#### 3.4.1 Synthesis of polyether-magnetite complexes

The synthesis of a trivinylsilyl-PPO-\textit{b}-PEO-OH is shown in Figure 3.3. A Zn$_3$[Co(CN)$_6$]$_2$ coordination catalyst (Impact™ from Bayer) was used for preparing the PPO blocks to avoid side reactions associated with base catalyzed ring-opening polymerizations of PO. It is well-known that significant amounts of undesired allyl-functional PPO form when hydroxide or alkoxide bases are utilized.\textsuperscript{143} \textsuperscript{1}H NMR of the trivinylsilyl-PPO-OH synthesized with the Impact™ catalyst revealed well-defined polymers without allyl endgroups. Trivinylsilyl-PPO-OH was then utilized as a macroinitiator for base catalyzed polymerization of EO, resulting in an amphiphilic, heterobifunctional trivinylsilyl-PPO-\textit{b}-PEO-OH diblock copolymer. 3-HPTVS was also utilized as the initiator for base catalyzed polymerization of EO to form trivinylsilyl-PEO-OH.

Ene-thiol additions of cysteamine hydrochloride on the trivinylsilyl-functional polyethers were performed to introduce amine functionality on the vinylsilyl termini. The reaction mixtures were deoxygenated prior to heating to afford complete conversion of the vinyl groups. After thiol addition, the polymers were washed with aqueous base to form free amines, resulting in triamine-functional polyethers. Figure 3.4 shows a representative \textsuperscript{1}H NMR spectrum that confirms the addition of cysteamine hydrochloride across the vinyl groups of a 7.2k M\textsubscript{n} trivinylsilyl-PEO-OH homopolymer.
Figure 3.3. Synthesis of a heterobifunctional trivinylsilyl-PPO-b-PEO-OH copolymer.

Triaammonium-functional diblock copolymers with varied PPO and PEO block lengths as well as a triammonium-PEO-OH homopolymer were utilized to prepare the magnetite-copolymer complexes for this study. Molecular weights and block lengths were analyzed by NMR and GPC with good agreement among the targeted values and both methods of analysis (Table 3.1). Molecular weight distributions of the PPO blocks were somewhat broader than for the PEO blocks. This arises from the heterogeneous nature of the coordination catalyst, and we are currently exploring synthetic avenues for narrowing those distributions. As expected, the PEO homopolymers that were synthesized by conventional anionic polymerizations had very narrow molecular weight distributions.
Figure 3.4. $^1$H NMR illustrates the disappearance of vinyl groups (1) on a 7.2kPEO-OH homopolymer after addition of cysteamine across the vinylsilane groups.

Table 3.1. Polyether molecular weights.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>$^1$H NMR PPO</th>
<th>$^1$H NMR PEO</th>
<th>GPC</th>
<th>PDI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2kPEO-OH</td>
<td>N/A</td>
<td>7,200</td>
<td>7,800</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>5.6kPPO-$b$-3.8kPEO-OH</td>
<td>5,600</td>
<td>3,800</td>
<td>10,100</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>5.6kPPO-$b$-7.2kPEO-OH</td>
<td>5,600</td>
<td>7,200</td>
<td>13,100</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>3.3kPPO-$b$-2.6kPEO-OH</td>
<td>3,300</td>
<td>2,600</td>
<td>5,400</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>3.3kPPO-$b$-4.8kPEO-OH</td>
<td>3,300</td>
<td>4,800</td>
<td>7,900</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

- PDI = Polydispersity index

3.4.2 Relationships between the amphiphilic nature of the polymer dispersion stabilizers, the density of chains on the magnetite surface and the colloidal properties of the complexes
One objective of this work has been to generate small clusters of magnetite-copolymer complexes that remain stable in dispersion. Clusters are desirable because larger particles may provide larger relaxivities and increase contrast enhancement in MRI imaging. To understand colloidal stability in physiological media, it was first helpful to examine the properties of these materials in water. Diameters of the complexes in water were measured via DLS every half-hour for 24 hours and the mean of the final 10 measurements were compared to those predicted by the model (Table 2). At high polymer compositions, the diameters of complexes with the PEO homopolymer and the first two diblocks described in Table 3.2 matched the model closely, indicating that these were dispersed as single particles. However, the DLS volume average diameters for complexes with those polymers became significantly higher than those predicted by the model as the polymer loading was reduced (suggesting clustering). For complexes with the triammonium-3.3kPPO-b-2.6kPEO-OH complexes where the block lengths were relatively short and the hydrophobic PPO was the dominant copolymer block, the volume averages were larger than the predicted values at all of the polymer loadings (33, 49 and 66% polymer). Thus, controlled clusters had formed with all of the complexes prepared with this particular copolymer. Surprisingly, these particles flocculated slightly until they reached some equilibrium diameter and then the sizes were stable (measured for >24 hours). This may be attributable to some mobility of the polymer chains on the surface or that the PPO layer of one particle generates an attractive hydrophobic force with the PPO layer of another particle. While the present model cannot account for the slight clustering, comparison of the DLS data to the model can identify when the flocculation takes place.
Table 3.2. A divergence in the experimental (measured over 24 hours) and predicted diameters of the complexes allows for estimating the lower limit of polymer loading where appreciable flocculation occurs. This phenomenon is more pronounced when comparing experimental and predicted volume average diameters.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Polymer Loading (%)</th>
<th>Dynamic Light Scattering</th>
<th>Distribution Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D_v* Nm</td>
<td>D_n** nm</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>39</td>
<td>43.2</td>
<td>33.8</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>45</td>
<td>38.5</td>
<td>30.7</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>65</td>
<td>45.5</td>
<td>37.2</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>33</td>
<td>46.9</td>
<td>34.7</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>51</td>
<td>39.3</td>
<td>31.0</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>68</td>
<td>42.2</td>
<td>34.9</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>31</td>
<td>45.1</td>
<td>35.6</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>49</td>
<td>39.1</td>
<td>29.5</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>67</td>
<td>34.0</td>
<td>27.4</td>
</tr>
<tr>
<td>3.3kPPO-b-2.6kPEO-OH</td>
<td>33</td>
<td>51.5</td>
<td>36.9</td>
</tr>
<tr>
<td>3.3kPPO-b-2.6kPEO-OH</td>
<td>49</td>
<td>39.0</td>
<td>29.8</td>
</tr>
<tr>
<td>3.3kPPO-b-2.6kPEO-OH</td>
<td>66</td>
<td>37.6</td>
<td>30.6</td>
</tr>
</tbody>
</table>

*Volume average diameter; **Number average diameter

Potential energy curves were generated for the complexes. Measured zeta potentials of the particles ranged from a minimum of -6 mV to a maximum of +6 mV. This indicated that surface charge contributed very little electrostatic stabilization and that steric stabilization was the dominating factor at close ranges (10^{-2} kT (electrostatic) vs. 10^2 kT (steric) at surface-to-surface separation distances <5 nm for the 5.6kPPO-b-3.8kPEO-OH complex with 70% polymer loading). Figure 3.5 shows potential energy curves for complexes with the triammonium-7.2kPEO-OH and triammonium-5.6kPPO-b-7.2kPEO-OH. Typically a potential energy maximum of >10kT is sufficient to prevent flocculation. As illustrated in Figure 3.5(B), the maximum for the diblock complex at 30% polymer loading is approximately 2kT and is approximately 28kT for the case of 50% polymer loading. At a volume fraction of 2.0 \times 10^{-5}, these loadings correspond to t_{1/2} values of 2.8 \times 10^{-3} and 2.2 \times 10^{6} seconds, respectively. Thus,
one would expect clustering to occur for the particles with 30% polymer, but not for those with 50% polymer. This is consistent with the data in Table 3.2, which shows clustering to an equilibrium diameter at 33% ($t_{1/2} = 4.2 \times 10^{-3} \text{ sec}$) but not at 51% polymer loading ($t_{1/2} = 3.8 \times 10^7 \text{ sec}$) as indicated by the number average diameters. The instability exhibited with low polymer loadings is even more evident in the data for the triammonium-5.6kPPO-\textit{b}-3.8kPEO-OH complex, which is reasonable since it has a much shorter hydrophilic block. The potential energy curves predict clustering for the case of 31% polymer loading ($t_{1/2} = 3.2 \times 10^{-3} \text{ sec}$) and this is confirmed by DLS. For the complex with 49% polymer ($t_{1/2} = 5.5 \times 10^5 \text{ sec}$), clusters with equilibrium diameters were observed via DLS. Although the $t_{1/2}$ seems high for clustering to occur in this case, some clustering is not altogether unexpected because the $t_{1/2}$ value is the measure of when half of the particles have formed doublets. Thus, when right at the transition from single particles to clusters (for example, 20% doublets instead of 50%), partial clustering would be observable via DLS even with the slightly elevated $t_{1/2}$. In addition, these $t_{1/2}$ calculations are extremely sensitive to polymer loading at the point of clustering and any slight error in the TGA measurement might cause inconsistencies between experimental and calculated sizes.

By contrast, the potential energy curves for the complexes coated with the triammonium-7.2kPEO-OH predict stability against clustering at 50% ($t_{1/2} = 3.0 \times 10^7 \text{ sec}$) and 70% ($t_{1/2} = 1.2 \times 10^{48} \text{ sec}$) polymer loadings, but not at 30% ($t_{1/2} = 1.8 \times 10^1 \text{ sec}$). These predictions are also consistent with the DLS results, as the volume and number average diameters do not significantly deviate from the predicted values until a small deviation is observed at 39% polymer (Table 3.2). The overall resistance to clustering for the homopolymer complexes compared to the 5.6kPPO-\textit{b}-7.2kPEO-OH and 5.6kPPO-\textit{b}-3.8kPEO-OH diblock complexes is most likely due to an increased
number of chains per particle for similar polymer loadings. Thus, it appears that the DLVO theory can reasonably predict the colloidal stabilities of these complexes. By contrast, for the complexes with the 3.3kPPO-\(b\)-2.6kPEO-OH copolymer, the experimental volume and number average diameters deviate from the predicted values for all polymer loadings while DLVO predicts resistance to clustering at high polymer concentrations (\(t^{1/2} = 5.2 \times 10^{17}\) and \(4.1 \times 10^{17}\) seconds for 70% and 50% polymer, respectively). This may be due to an additional hydrophobic interaction between the PPO blocks on separate complexes due to the relatively high composition of PPO combined with the short PEO block. In addition, we note that assumptions inherent in the calculation of the Hamaker constant may cause some inaccuracies in the DLVO predictions, especially since the Derjaguin approximation is not expected to fully apply for these small particles with highly curved brushes. However, these data indicate that experimental diameters can be compared to those predicted by the density distribution model when the calculated potential energy maximum is greater than about 10kT.
Number average diameters from DLS (measured immediately after the complexes were suspended in DI water and sonicated) and the model for complexes with the triammonium-7.2kPEO-OH, 5.6kPPO-b-7.2kPEO-OH and 5.6kPPO-b-3.8kPEO-OH are compared in Table 3.3. The potential energy curves for each of these complexes show maxima $>10kT$ and so clustering should be minimal. For these complexes, there is a maximum deviation of 23% (6.9 nm) and an average deviation of 8% (2.7 nm). This error is typical for DLS measurements in this size range. Thus, the density distribution model, with no adjustable parameters, can predict the number average diameters of complexes to within an average of 8% for non-clustered complexes. The
model also provides calculations of DLVO potential energy curves and half-lives for doublet formation that predict the polymer loading at which complexes will begin to exhibit clusters as the polymer composition is reduced. These correlate well with complex sizes measured by DLS. Deviation in this prediction occurs with the 3.3kPPO-\textit{b}-2.6kPEO-OH complexes, but this may be due to an additional attractive hydrophobic force or dispersion force not accounted for at present in the model.

### 3.4.3 Influence of material parameters on clustering of the complexes

While the radii of the complexes predicted from the model do not account for clusters, the model can be used to predict aggregation based on the stability ratios and half-lives for doublet formation ($t_{1/2}$). The sizes of these clusters were stable over a 24-hour measurement time and they did not sediment, so they could be easily compared. The $t_{1/2}$ values were calculated for the each of these complexes, and $\ln(t_{1/2})$ was plotted versus the intensity average diameters measured by DLS (24 hours after the particles were dispersed by sonication and then filtered) (\textbf{Figure 3.6}). Because the intensity average diameter is weighted heavily towards larger particles, it is ideal for probing cluster formation. An exponential function was fit to the data from the 5.6kPPO-\textit{b}-7.2kPEO-OH, 5.6kPPO-\textit{b}-3.8kPEO-OH and 3.3kPPO-\textit{b}-4.8kPEO-OH complexes. This fit indicates that the clustering behavior of these complexes can be accurately described by the $t_{1/2}$ values. This comparison is unique in the colloid literature to our knowledge and allows for predicting the cluster diameter based on the $t_{1/2}$ calculation. More importantly, deviations from the predicted diameter can be used to better understand interactions between specific complexes. Additionally, at higher polymer loadings, the number average diameters of each of these complexes matched those predicted by the model as described earlier. This indicates that by increasing the polymer loading, the complexes form discrete particles and clustering is eliminated.
Table 3.3. An average deviation of 8% was observed between experimental (measured immediately after dispersion by sonication followed by filtration) and predicted number average diameters for complexes that were predicted to be stable by DLVO theory.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Polymer Loading (%)</th>
<th>Chains per nm²</th>
<th>Model Dₙ (nm)</th>
<th>DLS Dₙ (nm)</th>
<th>Dev. (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2kPEO-OH</td>
<td>65</td>
<td>1.08</td>
<td>37.6</td>
<td>37.2</td>
<td>1%</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>48</td>
<td>0.55</td>
<td>33.0</td>
<td>32.4</td>
<td>2%</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>45</td>
<td>0.48</td>
<td>31.9</td>
<td>31.0</td>
<td>3%</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>44</td>
<td>0.47</td>
<td>32.0</td>
<td>35.9</td>
<td>11%</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>39</td>
<td>0.37</td>
<td>30.7</td>
<td>33.9</td>
<td>9%</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>30</td>
<td>0.25</td>
<td>28.5</td>
<td>28.7</td>
<td>1%</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>74</td>
<td>0.62</td>
<td>37.3</td>
<td>30.4</td>
<td>23%</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>68</td>
<td>0.46</td>
<td>34.9</td>
<td>31.6</td>
<td>10%</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>52</td>
<td>0.24</td>
<td>30.1</td>
<td>31.0</td>
<td>3%</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>52</td>
<td>0.24</td>
<td>30.1</td>
<td>27.6</td>
<td>9%</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>67</td>
<td>0.46</td>
<td>27.4</td>
<td>31.3</td>
<td>12%</td>
</tr>
</tbody>
</table>

By contrast, for the complexes with the more hydrophobic 3.3kPPO-b-2.6kPEO-OH copolymer that had the smaller block lengths, the exponential function only fit the experimental data at polymer loadings up to ~60%. The experimental diameters were significantly larger than the predicted values for loadings above 60% polymer. The DLS measurements (Table 3.2) show that complexes with this polymer formed clusters at all polymer loadings whereas the potential energy curves and t₁/₂ calculations indicated that clusters should not form above 55% polymer loading. For these complexes, increasing the polymer loading does not eliminate clustering at all. This suggests that clustering in those complexes at high polymer compositions may be due to a different mechanism that the current DLVO calculations do not account for. Since this deviation occurs in the complexes with very high compositions of copolymer, we hypothesize that the clustering in those materials may be largely attributable to the properties of the polymer. It may be that the hydrophobic PPO blocks on one nanoparticle are not sufficiently shielded from
neighboring nanoparticles by the short PEO blocks, and that PPO segments on multiple particles are associating through hydrophobic interactions. This is supported both by the deviation of the cluster diameters from the exponential fit, and the deviation of the experimental sizes from those predicted by the DLVO calculations (Table 3.2).

The accuracy of the exponential fit indicates that the $t_{1/2}$ is a useful tool for comparing complexes with different molecular weight polymers. This, along with the potential energy curves derived with the extended DLVO theory, provides evidence that the steric potential in Equation 3.16 is valid for these systems. Moreover, the deviation from the exponential fit for the 3.3$k$kPPO-$b$-2.6kPEO-OH copolymer provides insight into additional interactions that may be at play. The predictions of $t_{1/2}$ and the associated cluster diameter of a particle can aid in the design of

![Figure 3.6](image-url)

**Figure 3.6.** Intensity weighted diameters for the magnetite-copolymer complexes obtained from DLS vs. the calculated $\ln(t_{1/2})$: 5.6$k$kPPO-$b$-7.2kPEO-OH (diamonds), 5.6$k$kPPO-$b$-3.8kPEO-OH (triangles), 3.3$k$kPPO-$b$-4.8kPEO-OH (squares), and 3.3$k$kPPO-$b$-2.6kPEO-OH (empty circles).
copolymers required to obtain resistance to or formation of clusters. Similarly, such an approach can likely be extended to other colloidal systems.

3.4.4 Suitability of three ammonium ions as anchor segments for magnetite in water and PBS

Solution sizes of the magnetite-polymer complexes over time in DI water and PBS were examined with DLS to establish the conditions where these polymers had suitable binding affinities to be considered good dispersion stabilizers. Figure 3.7 shows colloidal sizes over time for magnetite coated with triammonium-5.6kPPO-b-7.2kPEO-OH. While the sizes were constant in water over 24 hours, some aggregation was observed with time in PBS, particularly at the lower polymer compositions. This behavior is attributed to some desorption of the polymers from the magnetite surface in PBS.

To understand the effects of PBS, the magnetite-copolymer complexes were dialyzed against water for 48 hours to remove any unbound polymer, against PBS for 24 hours to expose the complexes to phosphate, and then against water for another 24 hours to remove any unbound polymer that may have been displaced by the phosphate (as well as to remove phosphate salts from the liquid phase). Compositions of the complexes were measured by TGA after the water dialyses to examine whether any polymer had desorbed from the surface (Table 3.4). Each complex lost a significant amount of polymer after exposure to PBS for 24 hours. Thus, these experiments indicated that the cause of the size increases over time in PBS (as observed in Figure 3.7) versus constant sizes in water was primarily due to polymer desorption in PBS (and not caused by screening of electrostatic charge due to the ionic strength of PBS). This was confirmed by measuring sizes of the complexes in DI water with sodium chloride added at the same ionic strength as PBS. No increase in size over time was observed.
Figure 3.8 shows zeta potentials measured while varying the pH for an oleic acid-coated magnetite (Figure 3.8A) and a magnetite-7.2kPEO-OH complex (Figure 3.8B) in water and PBS. Note how the charge of both systems becomes markedly more negative in PBS, only passing through the zero net charge barrier at a pH of ~2.0-2.5. This pH corresponds with the lowest pK\textsubscript{a} of phosphoric acid. A similar change in charging behavior occurs for the other complexes.

Figure 3.7. DLS shows that the complexes with the triammonium-5.6kPPO-b-7.2kPEO-OH have long-term stability in DI water (A) but not in PBS (B). Percentages shown are % polymer in the complexes.

This, along with the dialysis data, indicates that the phosphate adsorbs relatively strongly onto the magnetite surface, even when a polymer brush is present. For all the complexes
examined, phosphate adsorption affects anchoring of the ammonium groups on the magnetite surface and causes some desorption of the polymer. In most cases, this desorption is sufficient to induce flocculation until the particles are large enough to form visible sediments within a few days. This indicates a limitation of the triammonium anchor group for a steric stabilizer when the complexes are to be used in physiological media.

Table 3.4. Dialysis experiments show that these polymer stabilizers desorb appreciably from the magnetite surface in PBS over 24 hours (after dispersion by sonication and filtration). No desorption was observed after dialysis against DI water.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Dialysis against water (% polymer)</th>
<th>Chains per nm²</th>
<th>Dialysis against PBS (% polymer)</th>
<th>Chains per nm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2kPEO-OH</td>
<td>50</td>
<td>0.58</td>
<td>39</td>
<td>0.37</td>
</tr>
<tr>
<td>7.2kPPO-OH</td>
<td>71</td>
<td>1.45</td>
<td>60</td>
<td>0.87</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>64</td>
<td>0.40</td>
<td>44</td>
<td>0.18</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>64</td>
<td>0.38</td>
<td>46</td>
<td>0.19</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>52</td>
<td>0.23</td>
<td>28</td>
<td>0.08</td>
</tr>
<tr>
<td>3.3kPPO-b-2.6kPEO-OH</td>
<td>62</td>
<td>0.64</td>
<td>51</td>
<td>0.41</td>
</tr>
</tbody>
</table>

It was of interest to understand how the rates of polymer desorption in PBS were influenced by the nature of the copolymer coatings. In particular, we wondered whether a relatively hydrophobic PPO sheath layer could hinder desorption significantly. The rates of size increase in PBS were approximately constant so the slopes of the lines were compared (e.g., in Figure 3.7). Table 3.5 summarizes the rates of increase for the complexes and predicts their cluster diameters at 24 hours. These data suggest that a PPO layer does slow the rate of desorption. When comparing the magnetite-7.2kPEO-OH complex with 45% polymer, the 5.6kPPO-b-7.2kPEO-OH complex with 68% polymer, and the 5.6kPPO-b-3.8kPEO-OH complex with 67% polymer—cases in which the chain densities are essentially equal ranging from 0.46-
0.48 chains nm$^{-2}$—it is apparent that the PPO layer plays a large role in increasing stability against flocculation. Notice that the 7.2kPEO-OH homopolymer complex, which is the most resistant to clustering in water, is the least stable in PBS. An order of magnitude more chains nm$^{-2}$ was required for the 7.2kPEO-OH complex to have the same slope as complexes prepared with the 5.6kPPO-b-7.2kPEO-OH copolymer (1.08 chains nm$^{-2}$ vs. 0.11 chains nm$^{-2}$, respectively).

**Figure 3.8.** The zeta potential of magnetite without polymer (A) and magnetite coated with polymer (B, triammonium-7.2kPEO-OH) changes and follows the same trend when measured in PBS compared to DI water. The lowest pK$_a$ of phosphoric acid is $\sim$2.2, which corresponds to the isoelectric point of both systems.
In addition, the sizes of the complexes with the 5.6kPPO- \textit{b} -7.2kPEO-OH are more stable than with the 5.6kPPO- \textit{b} -3.8kPEO-OH, indicating that the longer PEO brush (at similar numbers of chains nm\textsuperscript{2}) provides better stabilization in PBS. For the 5.6kPPO- \textit{b} -3.8kPEO-OH with 31% polymer, the flocculation rate is essentially equal to that of the 7.2kPEO-OH complex with 39% polymer. This is a much larger rate than for the 5.6kPPO- \textit{b} -7.2kPEO-OH complex with 33% polymer. This can be attributed to two competing factors. First, the hydrophobic PPO block slows desorption of the polymer from the surface, but does not prevent it. Second, the colloidal stability depends directly on the number of chains nm\textsuperscript{2}. For the 5.6kPPO- \textit{b} -3.8kPEO-OH complex with 31% polymer, the combination of the small hydrophilic block along with the large hydrophobic block and the relatively few chains on the particle surface contribute to the faster flocculation rate. For large PPO blocks, there is a limit to the total number of chains nm\textsuperscript{2} due to the size of the hydrophobic PPO. Thus, even though the data indicates that the PPO block somewhat protects the complexes from flocculation in PBS, larger PPO blocks result in fewer chains that can be adsorbed onto the magnetite surface.

Thus, our hypothesis is that reducing the PPO molecular weight might allow for better colloidal stability in PBS because the protective PPO layer would still be present, but this time with a higher number of polymer chains on the surface. Additionally, the deviation of the DLS data from the model and the exponential clustering fit indicate that hydrophobic forces are strong for the 3.3kPPO- \textit{b} -2.6kPEO-OH complexes. This is illustrated in Table 3.5, where the rate of flocculation for a 3.3kPPO- \textit{b} -2.6kPEO-OH complex with 48% polymer was zero. Thus, by reducing the molecular weight of the PPO block, colloidal stability in PBS can be obtained for greater than 24 hours.
Table 3.5. The change in diameter over time in PBS indicates that the PPO layer of the
diblock complexes hinders polymer desorption from the magnetite surface.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Polymer Loading (%)</th>
<th>Chains/nm²</th>
<th>Slope in PBS (nm/h)</th>
<th>Diameter increase Over 24 hours (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2kPEO-OH</td>
<td>39</td>
<td>0.37</td>
<td>28.8</td>
<td>691</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>45</td>
<td>0.48</td>
<td>21.6</td>
<td>518</td>
</tr>
<tr>
<td>7.2kPEO-OH</td>
<td>65</td>
<td>1.08</td>
<td>2.0</td>
<td>49</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>33</td>
<td>0.11</td>
<td>1.7</td>
<td>42</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>51</td>
<td>0.23</td>
<td>0.6</td>
<td>14</td>
</tr>
<tr>
<td>5.6kPPO-b-7.2kPEO-OH</td>
<td>68</td>
<td>0.46</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>31</td>
<td>0.10</td>
<td>29.3</td>
<td>703</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>49</td>
<td>0.22</td>
<td>1.0</td>
<td>23</td>
</tr>
<tr>
<td>5.6kPPO-b-3.8kPEO-OH</td>
<td>67</td>
<td>0.46</td>
<td>0.5</td>
<td>12</td>
</tr>
<tr>
<td>3.3kPPO-b-2.6kPEO-OH</td>
<td>52</td>
<td>0.42</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

3.5 Conclusions
A heterobifunctional triammonium-PEO-OH homopolymer and triammonium-PPO-b-
PEO-OH diblock copolymers were synthesized and adsorbed onto magnetite nanoparticles to
study their effects on colloidal stability in water and in PBS. A model for the copolymer-
magnetite complexes was employed which incorporated the magnetite core surrounded by a
hydrophobic PPO layer (treated as a non-solvated shell around the magnetite core) and a
hydrophilic PEO layer extending into solution.\(^9,10\) A polymer brush model was combined with
the magnetite core size distribution to predict complex size distributions that could be measured
by DLS. The model, in concert with a modified DLVO theory, was used to predict the polymer
loadings that were needed to prevent clustering. For systems that the DLVO theory predicted to
be single particles, the average deviation of the model from the DLS measurements for number
average diameters was only 2.7 nm (8%). By comparing the number average and volume average
diameters to the predicted values, the point of complex clustering could be experimentally
identified. For cases where clustering occurred, the small clusters reached an equilibrium
diameter and did not continue to grow. In water, the magnetite-PEO complexes were more resistant to clustering than their diblock counterparts at similar polymer loadings because the relatively hydrophobic PPO block restricts the total number of PEO chains that can be attached to the magnetite nanoparticle surface.

Half-lives for doublet formation ($t_{1/2}$) were calculated for the diblock complexes from the DLVO theory and were correlated with measured intensity average diameters (after 24 hours) in water by fitting the diameters and their $\ln(t_{1/2})$ values with an exponential function. For the complexes with the 3.3kPPO-$b$-2.6kPEO diblocks, the size deviations from the predictions were significantly higher at high polymer loadings, suggesting that copolymer-magnetite complexes with relatively long PPO blocks and short PEO blocks may exhibit additional hydrophobic interactions between the PPO blocks on separate complexes not yet accounted for in the DLVO theory. These hydrophobic interactions could be a key to designing small clusters of nanoparticles that have colloidal stability in aqueous media.

Stability in physiological media was examined through zeta potential measurements, dialysis, and long-term DLS measurements. While the sizes of the complexes were all constant in water for 24 hours, most of them showed a continual increase in diameter over time in PBS, indicating most of the polymers studied were partially displaced by phosphate salts in PBS. However, there was significantly less polymer desorption in PBS for the complexes having the amphiphilic diblocks relative to complexes with the triammonium-7.2kPEO-OH. Moreover, high levels of PEO chains nm$^{-2}$ are essential for colloidal stability in PBS as illustrated by the magnetite-triammonium-3.3kPPO-$b$-2.6kPEO-OH complex (0.42 chains nm$^{-2}$) that did not increase in size over time in water or PBS. For biological studies in physiological media, incorporation of a hydrophobic block to shield the magnetite surface from the phosphate salts and
the use of lower molecular weight PPO blocks to increase chain density may be essential for use of the triammonium anchor group. Current research in our laboratories is also exploring alternative anchoring groups for magnetite and the relative stabilities of those materials in PBS.

3.6 Acknowledgements

This material is based upon work supported in part by the Macromolecular Interfaces with Life Science IGERT of the NSF No. DGE-0333378 and by DMR-0312046. We are also grateful to Bayer Material Science, Inc. for their generous donation of the Impact™ coordination catalyst and for partial support of a student. Parts of this work were carried out in the Nanoscale Characterization and Fabrication Laboratory, a Virginia Tech facility operated by the Institute for Critical Technology and Applied Science. The authors thank Dr. Timothy St. Pierre, Dr. Robert G. Woodward and Dr. Ted S. Oyama for help with microscopy and XRD.
CHAPTER 4: Polyether-Magnetite Complexes for Enhanced Colloidal Stability in Physiological Media

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4.1 Introduction

Magnetite nanoparticles coated with biocompatible macromolecules have been of great interest due to a wide range of potential biomedical applications including drug delivery, treatment of detached retinas, cell separations and contrast enhancement agents for MRI. Poly(ethylene oxide) (PEO) has been one of the most widely used coatings for magnetite nanoparticles due to its low cytotoxicity, ability to mask foreign objects from the immune system, and FDA approval for in vivo applications. Despite the prevalence of PEO as a coating for magnetite, there is still a need to improve upon the binding efficacy of the polymer to the magnetite surface.

Functional anchor groups on polymers have been shown to aid in their adsorption to the surface of magnetite. Polymeric magnetite stabilizers containing carboxylic acid and alkylammonium anchor groups have been previously reported. Over the past few decades, significant effort has been devoted to the surface modification of magnetite with PEO-containing polymers to improve their biocompatibility, resist protein adsorption and increase their circulation time within the body. Previous work by others has shown the ability to stabilize magnetite nanoparticles with copolymers containing pendant carboxylic acid groups, as well as
Stability of these complexes in aqueous media was adequate, however, in physiological media the polymer-nanoparticle complexes showed instability.

This paper describes the synthesis of well-defined heterobifunctional poly(ethylene oxide) oligomers containing one anchor group (carboxylate, ammonium, or zwitterionic phosphonate) for adsorption onto magnetite nanoparticles. The relationships between the chemical nature of the anchor group and the colloidal stabilities of the PEO-magnetite complexes were studied in water and in PBS. Additionally, the influence of molecular weight on colloidal stability of the PEO-magnetite complexes with similar polymer loadings was investigated. Understanding the relative efficacies of anchor groups for magnetite can aid the design of polymer stabilizers that yield magnetite complexes with long-term physiological stability.

4.2 Experimental

4.2.1 Materials

Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), diethyl ether, diethyl vinyl phosphonate (97%), ethylene oxide (EO, 99.5+%), hexanes (HPLC grade), iron (III) acetylacetonate (Fe(acac)₃), mercaptoacetic acid (97+%), oleic acid (90%, technical grade), sodium iodide (99%), triethylamine (TEA, 99.5%), and 1.0 M vinylmagnesium bromide in THF were purchased from Aldrich and used as received. Tetrahydrofuran (THF, Optima Grade, EMD Science, 99.5%) was refluxed over sodium metal with benzophenone until the solution reached a deep purple, fractionally distilled, and deoxygenated just prior to use. Glacial acetic acid (EMD Science) was diluted with THF yielding a 2.0 M acetic acid solution. Naphthalene (Aldrich) was sublimed prior to use. Bromotrimethylsilane (TMS-Bromide, 97%) and mercaptoethanolamine
hydrochloride were purchased from Alfa Aesar and used as received. Ethanol (Decon Laboratories Inc.) was used as received. Methanol (HPLC grade), chloroform (HPLC grade), N,N’-dimethylformamide (DMF, Optima Grade), dichloromethane (HPLC grade), sodium bicarbonate, ammonium chloride, sodium chloride, and acetone (HPLC grade) were purchased from Fisher Scientific and used as received. 3-Chloropropylidimethylchlorosilane was purchased from Gelest and used as received. Dialysis tubing (25,000 g mol\(^{-1}\) MWCO and 1,000 g mol\(^{-1}\) MWCO) was obtained from Spectra/Por. Phosphate buffered saline 10X (PBS) was obtained from Lonza and diluted to appropriate concentrations.

### 4.2.2 Synthesis of 3-hydroxypropyldimethylvinylsilane (3-HPMVS)

3-HPMVS was prepared utilizing a modified procedure developed by Vadala et al.\(^{3,5}\) 3-Chloropropylidimethylchlorosilane (10.0 g, 0.06 mol) was syringed into a clean, flame-dried, two-neck roundbottom flask equipped with a stir bar under N\(_2\) purge. The reaction flask was placed in an ice bath and cooled to 0 °C. A 1 M solution of vinyl magnesium bromide (64.0 mL, 0.064 mol) in THF was slowly added to the flask over 30 min. The flask was allowed to warm to room temperature, and the mixture was stirred for 24 h. The reaction mixture was diluted with dichloromethane (100 mL), transferred to a separatory funnel and washed with a saturated aqueous ammonium chloride solution (150 mL), then the organic layer was further washed with aqueous sodium chloride (3 X 150 mL). Magnesium sulfate was added to the organic layer to remove any residual water, followed by vacuum filtration. Dichloromethane was removed under vacuum and the product was distilled at 100 °C, 0.8 Torr, yielding 3-chloropropylidimethylvinylsilane (8.91 g, 0.55 mol, 94% yield) (3-CPMVS).\(^1\)H NMR was used to confirm the quantitative addition of vinyl groups.
3-CPMVS (8.91 g, 0.55 mol) was placed in a 250-mL roundbottom flask equipped with a stir bar and condenser. In a separate roundbottom flask, sodium iodide (16.4 g, 0.11 mol) was dissolved in acetone (60 mL) and the solution was syringed into the flask. The mixture was heated at 56 °C for 24 h. Acetone was removed under vacuum and the product was dissolved in dichloromethane (100 mL) and vacuum filtered to remove the salt by-products. Dichloromethane was removed under vacuum and the product was distilled at 100 °C, 0.8 Torr, yielding 3-iodopropylidimethylvinylsilane (3-IPMVS, 13.4 g, 0.05 mol). \(^1\)H NMR confirmed the expected structure.

3-IPMVS (13.4 g, 0.05 mol) was placed in a 250-mL roundbottom flask equipped with a stir bar and condenser. DMF (20 mL) was added to the reaction flask followed by the addition of sodium bicarbonate (8.8 g, 0.10 mol) and DI water (5 mL). The mixture was heated to 100 °C for 24 h and the conversion of the alkyl iodide to an alcohol was monitored via \(^1\)H NMR. The reaction mixture was transferred to a separatory funnel and washed 3X with DI water to remove the excess sodium bicarbonate and DMF. The product was fractionally distilled at 90 °C, 0.8 Torr yielding 3-hydroxypropylidimethylvinylsilane (3-HPMVS, 7.4 g, 0.048 mol, 95% yield). \(^1\)H NMR confirmed the expected chemical structure.

**4.2.3 Synthesis dimethylvinylsilyl-functional PEO-OH (dimethylvinylsilyl-PEO-OH)**
An exemplary procedure for the synthesis of a dimethylvinylsilyl-PEO-OH is provided. An 8,300 g mol\(^{-1}\) M\(_n\) PEO oligomer was initiated with 3-HPMVS. A 300-mL, high-pressure Series 4561 Parr reactor was utilized for the polymerizations. EO (10.0 g, 0.23 mol) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath. THF (5 mL) was added to the reactor via syringe. A potassium naphthalide solution was prepared by charging naphthalene (14.1 g, 0.11 mol) into a 250-mL, flame-dried roundbottom flask equipped
with a glass stir bar. Dry THF (100 mL) was syringed into the flask to dissolve the naphthalene. Potassium metal (3.96 g, 0.10 mol) was added to the solution followed by a N₂ purge for 30 min. The solution was stirred overnight and titrated with 1 N HCl to determine the molarity of the potassium naphthalide solution, which was shown to be 0.95 M. An initiator solution consisting of 3-HPMVS (0.19 g, 1.29 mmol), THF (5 mL) and potassium naphthalide (1.26 mL of a 0.95 M solution in THF, 1.2 mmol) was prepared in a separate flame-dried, 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (0.66 mL of a 2.5 M solution in THF, 0.13 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N₂ for 1 h, then opened and its contents were transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The product was washed twice with DI water (2 X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

4.2.4 Functionalization of dimethylvinylsilyl-PEO-OH with carboxylic acid groups

An exemplary procedure for addition of a carboxylic acid group via ene-thiol free radical chemistry across the vinylsilyl endgroup is provided for an 8,300 g mol⁻¹ dimethylvinylsilyl-PEO-OH. Dimethylvinylsilyl-PEO-OH (1.0 g, 0.12 mmol) was charged to a 100-mL roundbottom flask equipped with a stir bar and dissolved in 2 mL of deoxygenated toluene. Mercaptoacetic acid (37.0 mg, 0.36 mmol) was syringed into the reaction flask followed by the addition of AIBN (9.2 mg, 0.06 mmol) dissolved in 0.5 mL of toluene. The mixture was deoxygenated for 10 min by sparging with N₂, then reacted at 80 °C for 24 h. The reaction mixture was dissolved in 200 mL of dichloromethane, then transferred to a separatory funnel and washed with DI water 3X to
remove the excess mercaptoacetic acid. The dichloromethane was removed via roto-evaporation and the resulting polymer was precipitated into cold diethyl ether. The polymer was dried at room temperature under vacuum for 24 h yielding 0.94 g of carboxylic acid-functionalized PEO.

4.2.5 Functionalization of dimethylvinylsilyl-PEO-OH with an ammonium group

Heterobifunctional polyethers with a terminal ammonium group (ammonium-PEO-OH) were obtained via the ene-thiol addition of mercaptoethylamine hydrochloride across the vinylsilane. In a characteristic procedure, an 8,300 g mol⁻¹ dimethylvinylsilyl-PEO-OH (2 g, 0.24 mmol), mercaptoethylamine hydrochloride (45.2 mg, 0.4 mmol), and AIBN (20 mg, 0.12 mmol) were dissolved in deoxygenated DMF (5 mL) in a 100-mL roundbottom flask equipped with a stir bar. The reaction was conducted at 70 °C for 24 h with stirring, then the reaction mixture was cooled to room temperature. DI water (100 mL) was added to the flask, and the mixture was transferred to a separatory funnel. Dichloromethane (200 mL) was added to the separatory funnel to extract the alkylammonium-functionalized polyether from the water layer. The dichloromethane layer was washed with a 1 N solution of sodium bicarbonate (3X), followed by 3 washes with DI water. The dichloromethane solution was concentrated under vacuum, and the ammonium-PEO-OH oligomer was precipitated into cold diethyl ether and dried at 25 °C under vacuum for 12 h yielding 1.9 g of product (95% yield).

4.2.6 Michael addition of diethyl vinyl phosphonate to ammonium-PEO-OH

A characteristic procedure for addition of a phosphonate group to an 8,300 g mol⁻¹ ammonium-PEO-OH is provided. An ammonium-PEO-OH oligomer (1.0 g, 0.12 mmol) was charged to a clean, flame-dried, 100-mL roundbottom flask equipped with a stir bar, and dissolved in ethanol (9 mL). TEA (0.20 mL, 0.14 mmol) was added to the reaction, followed by addition of diethyl vinyl phosphonate (0.26 mL, 0.14 mmol). The reaction was carried out at 70
°C for 24 h. The reaction mixture was diluted with DI water to obtain a 75:25 water:ethanol composition and placed in a 1,000 g mol\(^{-1}\) MWCO cellulose acetate dialysis bag and dialyzed for 24 h to remove excess diethyl vinyl phosphonate. The contents of the dialysis bag were transferred to a 100-mL roundbottom flask and lyophilized, yielding 0.91 g of diethyl phosphonate-functionalized PEO.

### 4.2.7 Hydrolysis of diethyl phosphonate-PEO-OH yielding phosphonic acid-PEO-OH

Phosphonic acid-PEO-OH was prepared from diethyl phosphonate-PEO-OH using a hydrolysis procedure adapted from Caplan et al.\(^{158}\) In a representative procedure, an 8,300 g mol\(^{-1}\) diethyl phosphonate-PEO-OH (0.80 g, 0.10 mmol) was charged to a clean, 100-mL roundbottom flask equipped with a stir bar and dissolved with 5 mL of dichloromethane. Trimethylsilyl bromide (0.032 mL, 0.24 mmol) was syringed into the reaction flask and stirred at room temperature for 24 h. Methanol (0.01 mL, 0.24 mmol) was added and stirred for 2 h to cleave the trimethylsilyl groups. Dichloromethane (50 mL) was added, and the mixture was washed 3X with DI water (100 mL each) in a separatory funnel. The dichloromethane layer was concentrated, then the oligomer was precipitated by pouring the mixture into cold diethyl ether. The polymer was dried at 25 °C under vacuum for 24 h yielding 0.77 g of phosphonic acid-PEO-OH.

### 4.2.8 Magnetite synthesis via reduction of Fe(acac)_3

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al.\(^{112}\) Fe (III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were charged to a 250-mL, three-necked roundbottom flask equipped with a water condenser and placed in a Belmont metal bath with an overhead stirrer with both thermostatic (+/- 1 °C) and revolution per minute control. The solution was sparged with N\(_2\) for 1 h. While stirring under N\(_2\),
the solution was heated at 100 °C for 4 h, then the temperature was increased to 205 °C at a rate of ~25 °C h⁻¹. Following 24 h at 205°C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone (100 mL each), then were dispersed in chloroform (20 mL) containing oleic acid (0.3 g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone (100 mL). The particles were dried under vacuum for 24 h at 25 °C. The composition of the particles obtained from thermogravimetric analysis (TGA) was 5% organic residue to 95% magnetite.

4.2.9 Adsorption of monofunctional polyether stabilizers onto magnetite nanoparticles

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50.0 mg) prepared as described above were dispersed in chloroform (10 mL) and charged to a 50-mL roundbottom flask. A monofunctional polyether (117.0 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The pH of each solution and the resulting mixture were approximately neutral. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂, and then the nanoparticles were precipitated in hexanes (300 mL). A magnet was utilized to collect the magnetite nanoparticles and free oleic acid was decanted with the supernatant. The complexes were dispersed in DI water (20 mL) using sonication for 30-60 s. The complexes were dialyzed against DI water (1 L) for 24 h using 25,000 g mol⁻¹ MWCO dialysis bags.
4.2.10 Characterization

$^1$H NMR spectral analyses of compounds were performed using a Varian Unity 400 NMR or a Varian Inova 400 NMR operating at 399.97 MHz.

An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

TGA measurements were carried out on the PEO-stabilized magnetite nanoparticles using a TA Instruments TGA Q500 to determine the fraction of each complex that was comprised of polymer. Each sample was first held at 110 °C for 10 min to drive off any excess moisture. The sample was then equilibrated at 100 °C and the temperature was ramped at 10 °C min$^{-1}$ to a maximum of 700 °C in a nitrogen atmosphere. The mass remaining was recorded throughout the experiment. The mass remaining at 700 °C was taken as the fraction of magnetite in the complexes. TGA was used to determine the polymer loading for each complex.

DLS measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Intensity, volume and number average diameters were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters. For DLS analysis, the dialyzed complexes dispersed in DI water were diluted to ~0.05 mg mL$^{-1}$ and filtered through a Whatman Anotop 100-nm alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of $1.3 \times 10^{-5}$ to $2.2 \times 10^{-5}$ depending on the polymer loading on the magnetite. In experiments where PBS was added, 0.1 mL of 10X PBS was mixed with 0.9
mL of the 0.05 mg mL\(^{-1}\) complex solution, then the solution was filtered through a 100-nm alumina filter into a clean polystyrene cuvette. Each sample was analyzed immediately following filtration and re-measured every 30 min over 24 h.

A 7T MPMS Squid magnetometer (Quantum Design) was used to determine magnetic properties. Hysteresis loops were generated for the magnetite nanoparticles at 300K and 5K. Fe concentration of the magnetite was determined chemically by acid digestion followed by ICP-AES analysis. These concentrations were in good agreement with TGA values.

### 4.3 Results and Discussion

One primary issue regarding the structures of magnetite-polymer complexes for biomedical applications is the long-term colloidal stability of dispersions of such complexes in physiological media.\(^7\,^{150,151}\) The aim of this study has been to compare the binding efficacies of PEO oligomers with different functional anchoring groups to magnetite, particularly in the presence of phosphate salts found in physiological media. PEO oligomers with one terminal carboxylate, ammonium or a zwitterionic phosphonate were adsorbed onto magnetite, and the complexes were compared. Knowledge of which binding groups remained stably bound to the magnetite nanoparticles then allowed us to examine the colloidal stabilities of these magnetite-polymer dispersions in light of the molecular weights and chain densities of the bound oligomers on the magnetite surfaces.

#### 4.3.1 Synthesis of PEO oligomers with different functional endgroups for binding to magnetite

3-HPMVS was used as a versatile initiator for the living anionic polymerization of EO to produce heterobifunctional oligomers that could be post-functionalized with the different
chemical groups to adsorb onto the magnetite nanoparticles (Scheme 4.1). This approach allowed for utilizing the same oligomers for comparing dispersion properties with the only difference being the functional anchor groups. Potassium naphthalide was reacted with 3-HPMVS to form the alkoxide for polymerizing EO, and the number of moles of 3-HPMVS relative to EO controlled the molecular weight. A small deficiency of the naphthalide relative to 3-HPMVS ensured that only the alkoxide (and not residual naphthalide) initiated the chains, and any remaining alcohol chains transferred with the growing PEO chains throughout the reaction. It is noteworthy that these polymerizations were terminated with acetic acid prior to opening the reaction vessel to limit any unwanted oxidative side reactions.


Dimethylvinylsilyl-PEO-OH oligomers with targeted molecular weights of 3,000 and 8,000 g mole\(^{-1}\) were prepared by adjusting the 3-HPMVS to monomer ratios, and the materials were characterized by \(^1\)H NMR and GPC (Table 4.1). Figure 4.1 shows a representative NMR spectrum of a 2,900 g mole\(^{-1}\) dimethylvinylsilyl-PEO-OH. Number average molecular weight was determined by comparing the integral ratios of the resonances corresponding to the methylene groups in the initiator (labeled 3 and 4) to the repeat unit of ethylene oxide labeled 6. GPC analysis of the dimethylvinylsilyl-functional polyethers revealed symmetric monomodal
peaks with molecular weight distributions of less than 1.1, which is indicative of living anionic polymerizations (Figure 4.2). Good molecular weight agreement was found between both methods of analysis.

![Figure 4.1. 1H NMR of a 2,900 g mole⁻¹ dimethylvinylsilyl-PEO-OH.](image)

Table 4.1. Molecular weights of dimethylvinylsilyl-PEO-OH.

<table>
<thead>
<tr>
<th>Targeted Molecular Weight</th>
<th>$M_n$ (g mole⁻¹)</th>
<th>PDI'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'H NMR</td>
<td>GPC</td>
</tr>
<tr>
<td>3,000 g mole⁻¹</td>
<td>2,900</td>
<td>3,100</td>
</tr>
<tr>
<td>8,000 g mole⁻¹</td>
<td>8,300</td>
<td>7,900</td>
</tr>
</tbody>
</table>

*PDI = Polydispersity Index
Ene-thiol additions were utilized to introduce carboxylic acid and ammonium endgroup-functionality onto the monofunctional polyethers (Scheme 4.2). The vinylsilyl functionality is unusual among vinyl groups in that it does not polymerize readily by free radical reactions, and thus it is seemingly an ideal substrate for ene-thiol functionalization reactions because polymerization does not compete. Nevertheless, an excess of the thiol relative to vinylsilane, as well as more than the "normal" amount of AIBN initiator, was utilized for these reactions to ensure quantitative addition.

Scheme 4.2. Ene-thiol addition of 2-mercaptop ethylamine hydrochloride (R₁) and mercaptoacetic acid (R₂) to dimethylvinylsilyl-PEO-OH.
Addition of mercaptoacetic acid to dimethylvinylsilyl-PEO-OH was monitored by $^1$H NMR. Complete disappearance of the vinyl group at approximately 6 ppm indicated stoichiometric conversion to a monocarboxylic acid-PEO-OH as shown in Figure 4.3. Additionally, the appearances of the methylene peaks labeled 1-3 in the spectrum indicated addition of mercaptoacetic acid. Comparison of the resonance integrals corresponding to the methylene peaks of the thiol (1-3) to the methylene peak labeled 5 confirmed quantitative addition of mercaptoacetic acid.

![Figure 4.3](image)

**Figure 4.3.** Ene-thiol addition of mercaptoacetic acid to a 2,900 g mole$^{-1}$ polyether yielding a carboxylic acid functionalized-PEO (HOOC-PEO-OH).

$^1$H NMR was utilized to monitor the ene-thiol addition of 2-mercaptopetlyamine hydrochloride (Figure 4.4) by observing the complete disappearance of the vinyl group and appearances of methylene peaks labeled 1-4 in the spectrum. Comparison of integrations confirmed quantitative addition of ammonium functionality to the dimethylvinylsilyl-PEO-OH.
Ammomium-PEO-OH was used as a precursor for the formation of zwitterionic phosphonate-functional polyethers. The ammonium endgroup was then reacted with triethylamine to afford a free amine endgroup. Diethyl vinyl phosphonate was added to the amine group via Michael addition, yielding diethylphosphonate-PEO-OH (Scheme 4.3). A slight molar excess of triethylamine was added to maintain basic reaction conditions, aiding in the addition of the diethyl vinyl phosphonate.
Scheme 4.3. Michael addition of diethyl vinyl phosphonate to ammonium-PEO-OH.

$^1$H NMR was used to monitor the addition of diethyl vinyl phosphonate to ammonium-PEO-OH (Figure 4.5). The appearances of two methylene peaks corresponding to the converted vinyl group of the phosphonate reactant were observed as peaks 3 and 4 in the spectrum of the product. Quantitative addition of diethyl vinyl phosphonate was determined by comparing the integrations of peaks 1-4 to the integration of the methylene peak labeled 10.

Figure 4.5. $^1$H NMR of a 2,900 g mole$^{-1}$ diethylphosphonate-PEO-OH.
For binding to magnetite, the ethyl phosphonate groups were cleaved using bromotrimethylsilane (TMS-Br). Addition of TMS-Br yielded an intermediate bistrimethylsilyl ester with an alkyl halide by-product. In the methanolysis step of the reaction, the alcohol cleaved the silyl ester yielding phosphonic acid (Scheme 4.4). A zwitterionic endgroup was afforded in the phosphonate-PEO-OH product at a pH less than 10, due to the negative charge of the phosphonic acid and the protonated secondary amine.

Scheme 4.4. Cleavage of diethylphosphonate-PEO-OH to zwitterionic phosphonate-PEO-OH.

\(^1\)H NMR was used to confirm the methanolysis of the diethylphosphonate-PEO-OH (Figure 4.6). In addition to the disappearance of the ethyl resonances in the spectrum, there was a shift upfield of the methylene group adjacent to the phosphorus atom (~1.6 ppm). Integration of the peaks in the spectrum show quantitative cleavage of the ethyl phosphonate groups in the product.
PEO-magnetite complexes with similar polymer loadings were formed with the two molecular weight PEO oligomers, each containing the various endgroups, and the complexes were dialyzed to remove any unbound PEO. Table 4.2 lists the series of complexes, polymer loadings determined by TGA after dialysis and number weighted average diameters as measured by dynamic light scattering (DLS). The compositions of the PEO-magnetite complexes were in close agreement with the targeted 30 wt% magnetite:70 wt% polyether composition. The PEO-magnetite complexes with the ammonium and zwitterionic phosphonate anchor groups show approximately the same number weighted average diameter via DLS, while the complexes with the carboxylate anchor group are significantly smaller. It should be noted that the diethylphosphonate-PEO-OH polymers were soluble in aqueous media. However, the resulting magnetite complexes using the diethylphosphonate-PEO-OH stabilizers were not stable. The
magnetite nanoparticles were magnetically characterized via superconducting quantum interference device. Hysteresis loops revealed superparamagnetic behavior and a saturation magnetization of 80 emu/g of magnetite.

### Table 4.2. PEO-magnetite complex compositions.

<table>
<thead>
<tr>
<th>PEO Molecular Weight (g mole(^1))</th>
<th>Anchor Group</th>
<th>Polymer Loading After H(_2)O Dialysis (Wt %)</th>
<th>(D_n) (nm)</th>
<th>Polymer Loading After PBS Dialysis (Wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,900</td>
<td>Carboxylate</td>
<td>65</td>
<td>19.9</td>
<td>41 ± 1.4</td>
</tr>
<tr>
<td>8,300</td>
<td>Carboxylate</td>
<td>69</td>
<td>24.5</td>
<td>45 ± 1.4</td>
</tr>
<tr>
<td>2,900</td>
<td>Ammonium</td>
<td>66</td>
<td>26.8</td>
<td>41 ± 0.7</td>
</tr>
<tr>
<td>8,300</td>
<td>Ammonium</td>
<td>79</td>
<td>31.7</td>
<td>43 ± 0.7</td>
</tr>
<tr>
<td>2,900</td>
<td>Zwitterionic Phosphonate</td>
<td>63</td>
<td>26.2</td>
<td>65 ± 1.4</td>
</tr>
<tr>
<td>8,300</td>
<td>Zwitterionic Phosphonate</td>
<td>71</td>
<td>39.6</td>
<td>68 ± 0.7</td>
</tr>
</tbody>
</table>

#### 4.3.2 Colloidal stabilities of the PEO-magnetite nanoparticle dispersions in water and PBS

Stabilities of the PEO-magnetite complexes against flocculation in DI water and PBS were examined using DLS. The relative efficacies of the three anchor groups in both media were studied by measuring the PEO-magnetite complex diameters every 30 minutes for 24 hours. Figure 4.7 shows the diameters of the complexes containing the PEO oligomers with the ammonium anchor group over time in DI water (A) and PBS (B). Intensity weighted diameters are reported due to their sensitivity to the presence of aggregates (since they scale with radii to the 6th power), so that any agglomeration with time is magnified.
DLS data of the ammonium-PEO-OH-magnetite complexes do not show significant changes in diameters of the complexes over 24 hours in water, while large increases in the sizes were observed for the complexes in PBS. Based on the terminal velocity of a sphere calculation (Eq. 4.1) in solution, a magnetite particle should settle to the bottom of the DLS cuvette when an aggregate diameter of ~700-1000 nm is reached.\textsuperscript{153}

\begin{equation}
U = \frac{2}{9} \frac{r^2 g (\rho_{mag} - \rho_{solvent})}{\mu_{solvent}}
\end{equation}

Eq. 4.1

Here, $U$ is the terminal velocity of the particle, $g$ is the gravitational constant, $r$ is the particle radius, $\rho_{mag}$ is the density of the magnetite, $\rho_{solvent}$ is the density of the solvent, and $\mu_{solvent}$ is the viscosity of the solvent at 25 °C. Thus, the ammonium-PEO-OH-magnetite complexes after ~200 minutes in PBS are likely on the verge of sedimentation and the sizes measured by DLS do not represent complexes that are at equilibrium in terms of size. Moreover, sediment was visually observed for the ammonium-PEO-OH-magnetite complexes in PBS after 24 hours.

\textbf{Figure 4.8} shows the diameters of the complexes containing the carboxylate anchor group over time in DI water (A) and PBS (B). Figure 8(C) shows the 2,900 g mole$^{-1}$ complex in PBS on
a smaller scale. These complexes showed no increase in intensity average diameter in DI water over 24 hours. By contrast, flocculation was observed for the 8,300 g mole\(^{-1}\) carboxylate-PEO-OH-magnetite complex in PBS over time. Although the complex with the 2,900 g mole\(^{-1}\) PEO oligomer did not visibly settle in PBS, aggregation to \(~\)100 nm in intensity weighted diameter was observed with an equilibrium diameter reached after \(~\)5 hours.

**Figure 4.8.** DLS intensity weighted diameters of the 2,900 (open circle) and 8,300 g mole\(^{-1}\) (black diamond) of the carboxylate-PEO-OH-magnetite complexes in DI water (A) and PBS (B) over 24 hours. A magnified plot of the 2,900 g mole\(^{-1}\) carboxylate-PEO-OH-magnetite complex in PBS is shown in (C).

The relative stability of the complexes in DI water suggests that polymer desorption from the magnetite is the cause of flocculation in PBS. Based on previous work, we believe that the phosphate salts from the PBS are displacing the polymer anchor groups.\(^{150}\) This reduces steric
repulsion and promotes flocculation of particles due to pair-pair van der Waal’s interactions. **Figure 4.9** illustrates the adsorption of phosphate salts onto the magnetite surface.

![Zeta Potential vs pH for Phosphate Buffer Saline (PBS) and DI Water](image)

**Figure 4.9.** Zeta potential shows that phosphate salts from the PBS adsorb to the magnetite surface.

PEO molecular weight has an appreciable effect on the behavior of these complexes in PBS. A possible explanation for this difference in behavior is the number of polymer chains anchored to the magnetite nanoparticle surfaces for each complex. Each complex has approximately the same polymer loading (~65 wt %), resulting in more total chains for the 2,900 g mol\(^{-1}\) complexes (~3.0 chains nm\(^{-2}\)) than the 8,300 g mol\(^{-1}\) complexes (~1.1 chains nm\(^{-2}\)). A denser polymer brush may provide greater stability in PBS for the smaller molecular weight PEO complexes.

This data also indicates that while complexes containing both carboxylate and ammonium anchor groups are susceptible to polymer desorption, they show significant differences in flocculation behavior for the same PEO molecular weight and polymer loading. DLS data presented in **Table 4.2** shows that the complexes with carboxylate anchor groups have significantly smaller number weighted average diameters than their ammonium counterparts. The
smaller diameter for the carboxylate anchor group complexes indicates a higher local density of polymer near the magnetite surface. Shorter, denser polymer brushes provide greater stability against flocculation for different molecular weights, and a similar phenomenon appears to be happening when comparing the ammonium and carboxylate anchor groups.

**Figure 4.10** shows the intensity weighted diameters of the complexes containing the zwitterionic phosphonate anchor groups over time in DI water (A) and PBS (B). **Figure 4.10(C)** shows the intensity weighted diameter of the 8,300 g mole⁻¹ PEO magnetite-complex over time in DI water and a 0.17 M NaCl aqueous solution (same ionic strength as the PBS used). The complex with the 2,900 g mole⁻¹ zwitterionic phosphonate-PEO-OH stabilizer was relatively stable in DI water and the corresponding complex with the 8,300 g mole⁻¹ stabilizer showed only a slight increase in diameter. Increased stability was observed for the 8,300 g mole⁻¹ zwitterionic phosphonate complex in NaCl aqueous solution. This has two major implications: 1) the ionic strength of the PBS is not causing flocculation of the particles, as the NaCl solution has the same ionic strength, and 2) increased stability of the 8,300 g mol⁻¹ complex over time in NaCl versus DI water indicates an electrostatic attraction between individual complexes causing flocculation in DI water. Through addition of the NaCl (or PBS), the Debye length of this electrostatic attraction is significantly reduced, preventing flocculation.
Figure 4.10. DLS intensity weighted diameters of the 2,900 (open circle) and 8,300 g mole\(^{-1}\) (black diamond) zwitterionic phosphonate-PEO-OH-magnetite complexes in DI water (A) and PBS (B) over 24 hours. (C) is the intensity weighted diameter of the 2,900 g mole\(^{-1}\) zwitterionic phosphonate-PEO-OH-magnetite complex in DI water (black diamond) and 0.17M NaCl (open circle). (D) is the intensity weighted diameter of the 8,300 g mole\(^{-1}\) zwitterionic phosphonate-PEO-OH-magnetite complex in DI water (black diamond) and 0.17M NaCl (open circle).

The stabilities of the dispersions in PBS that were prepared with the zwitterionic phosphonate-PEO-OH were significantly improved over the analogous materials with either the carboxylate or ammonium anchor groups. In contrast to any of the carboxylate- and ammonium-stabilized materials, these complexes with the phosphonate endgroups were significantly smaller in size, indicating their resistance against aggregation in PBS. While these materials did increase slightly in size over the 24 hours studied, the rates of increase and the amounts of increase, were small. The complexes with the larger 8,300 g mole\(^{-1}\) PEO showed similar small amounts of slow
flocculation in both water and PBS, thereby suggesting the phosphate salts in the medium had little effect.

The mechanism of binding for the anchor groups to the surface of magnetite is uncertain, and future investigation may elucidate the reasons for the observed trends. However, the relative stability of these zwitterion phosphonate complexes in PBS was higher than the other complexes with comparable molecular weights. The trend of anchor group efficacy for PEO-magnetite complexes in PBS appears to be zwitterionic phosphonate >> carboxylic acid > ammonium.

4.4 Conclusions
In this paper, we report that polyether-magnetite complexes wherein PEO oligomers are absorbed through carboxylic acid or ammonium anchor groups exhibit time-dependent colloidal instability in phosphate buffered saline (PBS), whereas complexes prepared with a PEO having a terminal zwitterionic phosphonate anchoring group are stable. The colloidal instabilities in the former cases are attributed to polymer desorption from the surface of the magnetite nanoparticles in the presence of phosphate salts.150

4.5 Acknowledgements
The authors are grateful for the financial support from NSF under contract DMR. They are also grateful for measurements of the magnetic properties of these materials by M. R. J. Carroll and T. G. St. Pierre in the School of Physics, University of Western Australia.
**CHAPTER 5: Synthesis and Characterization of a One-part PDMS-Magnetite Nanoparticle Fluid**

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**5.1 Introduction**

Ferrofluids are magnetically active fluids. They were originally developed in the 1940s and consist of very small magnetic particles suspended in a carrier fluid. They differ from earlier suspensions of magnetic particles in that the ferrofluid suspensions are stable even in the presence of large magnetic fields and field gradients. These fluids show a variety of physical phenomena that have generated both scientific interest and practical applications over the past ~60 years. Their use in a series of engineering applications such as audio speakers and as vacuum and rotational seals has become commonplace. There are also other potential applications such as ferrofluid based actuators, electromagnetic micropumps, and fluid-based valves and sealing systems.159-161 In addition, we have been working for some time to develop biocompatible ferrofluids† for use in biotechnology; where applications may include drug carriers, fluids for

† In this work a biocompatible ferrofluid is defined as one in which the properties of a magnetically active fluid are important as compared to biocompatible magnetic micro- and nanoparticles.
remote treatment of arterial aneurysms, or liquid ball valves for temporarily closing nanometer to micron size openings.

Ferrofluids are generally made up of three components.

1) Superparamagnetic nanoparticles - These are small magnetic particles generally in the range of 5 to 20 nm in size that are superparamagnetic. These materials have no magnetic moment in the absence of a field, but have a very high moment even in relatively low applied fields. Superparamagnetism is a result of the small particle size, and having superparamagnetic particles is important for the generation and maintenance of a stable ferrofluid.

2) A coating on the nanoparticles - The particles are coated with a small molecule or polymer to generate stable fluids. These coatings can be organic acids such as oleic or citric acid, or polymers based on, for example, poly(ethylene oxide) or polydimethylsiloxane that adsorb onto the particle surfaces through interactions of functional groups on the coating material and the nanoparticles. The coatings reduce the attractive potential between the magnetic particles through electrostatic and/or steric repulsion. The coating and the nanoparticles form a complex that becomes one integral component.

3) Carrier fluid - The final component is usually a carrier fluid in which the coated particles are suspended.

For several years we have been working on the development of biocompatible ferrofluids suitable for a novel treatment for retinal detachment. In this treatment, the ferrofluid is used to apply a force from inside the eye to seal a small tear in the retina and hence prevent seepage of fluid behind the retina. The fluid is injected into the eye in opposition to a small permanent magnet inserted at the back of the eye. The permanent magnet attracts the ferrofluid toward the back of the eye where it contacts the retina and presses the retina back into
place. The broader impact on healthcare of this application alone is significant. Retinal detachment is a leading cause of blindness, and currently available treatments fail in as many as 1/3 of complicated retinal detachment patients, resulting in partial or complete loss of vision for several million people worldwide. The fluids for this application are magnetite-based ferrofluids coated with polydimethylsiloxane (PDMS), dispersed in a PDMS oligomer as the carrier fluid.

In this paper we will introduce new ferrofluids based on PDMS-coated magnetite that differ significantly from other ferrofluids in that they do not contain carrier fluids. This avoids any possibility of the nanoparticles settling out of a dispersion. The coated magnetite particles are themselves viscous fluids at ambient temperature due to the high flexibility (i.e., low $T_g$s) of the PDMS coatings. We will describe the synthesis and characterization of these "one-part ferrofluids" and illustrate issues regarding their fluid behavior in these systems.

5.2 Experimental

5.2.1 Materials

Hexamethylcyclotrisiloxane ($D_3$, Gelest, Inc., 98%) was dried over calcium hydride and sublimed prior to use. Cyclohexane (Fischer Scientific, HPLC grade) was stirred with concentrated sulfuric acid for 48 h, washed with deionized water until neutral and dried over magnesium sulfate. The cyclohexane was then stirred over calcium hydride, fractionally distilled under vacuum, stored over sodium in a nitrogen atmosphere, and distilled just prior to use. Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. Toluene (Burdick and Jackson, 99.9%) was used as received. 2,2’-Azobisisobutyronitrile (AIBN, 98%), n-butyllithium (2.5 M solution in hexanes) and mecaptoacetic acid (97%) were purchased from
Aldrich and used as received. Trivinylchlorosilane (Gelest Inc., 95%) and trimethylchlorosilane (Gelest Inc., 99%) were used as received. Ammonium hydroxide (VWR International) was diluted with Millipore water to yield a 50/50 v/v solution and deoxygenated with nitrogen just prior to use. Iron (III) chloride hexahydrate (≥ 98%) and iron (II) chloride tetrahydrate (99%) were obtained from Sigma-Aldrich and were ground into fine powders and stored under nitrogen prior to use. Hydrochloric acid (EMD, 12.1 M) was added to deionized (DI) water to yield a 3.0 M solution. DI water was deoxygenated just prior to use. Dichloromethane was obtained from EMD and used as received. Iron granules (Alfa Aesar, 1-2 mm, 99.98%) were washed repeatedly with a variety of solvents to remove any coating on the surface and subsequently dried overnight in a vacuum oven at 40 °C. The iron granules (6 g) were then placed into a 3 mL syringe packed with glass wool to obtain magnetic separation columns. NdFeB doughnut-shaped magnets were purchased from Engineered Concepts and had an outer diameter of 2.54 cm, an inner diameter of 1.26 cm and were 0.65 cm thick. Magnetic separation columns were prepared by magnetizing the iron-granule-packed syringe with the doughnut-shaped magnets.

THF, toluene, acetone (Fischer Scientific, HPLC grade), THF-d₈ (Aldrich, 99.5 atom % D), toluene-d₈ (Aldrich, 99.6 atom % D) and acetone-d₆ (Aldrich, 99.9 atom % D) were used as received for SANS sample preparation.

5.2.2 Synthesis of trivinylsilyl-terminated PDMS

The synthesis of a targeted 3,000 g mol⁻¹ trivinylsilyl-terminated PDMS is provided. Other molecular weights were prepared in an analogous manner with appropriate ratios of initiator to monomer to control chain length. D₃ (26.5 g, 0.119 mol) was sublimed into a flame-dried roundbottom flask containing a magnetic stir bar and purged with nitrogen. Cyclohexane (26 mL) was added to the flask via syringe and the D₃ monomer was dissolved at room temperature. n-
Butyllithium (2.5 M, 3.18 mL, 0.00795 mol) was added to the reaction flask via syringe and the solution was stirred for 1 h, followed by the addition of THF (10 mL) to the solution as a reaction promoter. The living anionic polymerization was monitored using $^1$H NMR, and at ~90% conversion of monomer (~18 h), the polymer was terminated with an excess of trivinylchlorosilane (1.72 mL, 0.0119 mol). The solution was stirred overnight and then concentrated under vacuum at 40 °C. The product was dissolved in 200 mL of dichloromethane, washed three times with DI water (100 mL each), concentrated under vacuum and precipitated into methanol (300 mL). The recovered trivinylsilyl-terminated PDMS oligomer was dried under vacuum at 80 °C overnight. $^1$H NMR confirmed the expected chemical structure.

5.2.3 Functionalization of trivinylsilyl-terminated PDMS with mercaptoacetic acid

A representative procedure for functionalizing the PDMS oligomers on one end only is provided below. Other molecular weight oligomers were functionalized in similar reactions. A PDMS oligomer with three carboxylic acid groups on one terminus was prepared via a thiol-en e addition of mercaptoacetic acid across the vinylsilane endgroups. A 3,100 g mol$^{-1}$ trivinylsilyl-terminated PDMS (10 g, 0.01 mol vinyl) was added to a flame-dried roundbottom flask containing a magnetic stir bar, and dissolved in deoxygenated toluene (10 mL). The reaction mixture was sparged with nitrogen for 2 h to remove oxygen, and then AIBN (2.5 x 10$^{-3}$ g, 1.7 x 10$^{-4}$ mol) and mercaptoacetic acid (1.11 mL, 0.016 mol) were added into the flask. The reaction mixture was sparged with nitrogen for 0.5 h and then heated at 80 °C with stirring for 3 h. $^1$H NMR was used to observe the quantitative disappearance of the vinyl proton peaks (~6 ppm), indicating completion of the thiol-ene functionalization reaction. The reaction mixture was concentrated under vacuum at 60 °C, and the product was dissolved in methanol (10 mL). DI water was added dropwise to the solution until a white solid precipitate formed, which was
collected via vacuum filtration. This precipitation was repeated 3X, and the recovered polymer was dried under vacuum overnight at 80 °C.

5.2.4 Synthesis of non-functional PDMS as a carrier solvent for a PDMS-magnetite nanoparticle fluid

A 6,000 g mol⁻¹ PDMS was synthesized using living anionic polymerization of D₃ as described above. Once the growing polymer chains reached the desired molecular weight as observed with ¹H NMR, the polymer was terminated with an excess of trimethylchlorosilane. The polymer isolation procedure for the non-functional PDMS was the same as for the trivinylsilyl-terminated PDMS.

5.2.5 Synthesis of PDMS-stabilized magnetite complexes

Synthesis of magnetite nanoparticles and subsequent adsorption of a representative carboxylate-functional PDMS dispersion stabilizer onto the nanoparticle surfaces was achieved via the following procedure. The experimental conditions describe a method to obtain a PDMS stabilizer-magnetite complex comprised of ~30 wt% magnetite and ~70 wt% PDMS as the dispersion stabilizer. Magnetite nanoparticles were prepared using a chemical co-precipitation of iron salts. Iron (III) chloride hexahydrate (3.50 g, 0.013 mol) and iron (II) chloride tetrahydrate (1.28 g, 0.0064 mol) were weighed into separate roundbottom flasks, and each was dissolved in 20 mL of deoxygenated water. The two iron salt solutions were then added to a 500-mL, three-necked, roundbottom flask fitted with an Ultra-Turrax T25 Digital Homogenizer, a pH electrode and a nitrogen purge. The iron salts solution was stirred at 13,000 rpm with the homogenizer and the ammonium hydroxide solution (~20 mL) was added via syringe until the rapidly stirring solution turned black and reached a pH of 9-10. The PDMS dispersion stabilizer (3.5 g) was dissolved in dichloromethane (60 mL), and this solution was added to the basic magnetite
dispersion and stirred for 30 min. Aqueous HCl (3.0 M) was then slowly added until a slightly acidic pH was obtained (~12 mL was required to reach pH 5-6). The heterogeneous dispersion was stirred for 1 h, then transferred to a separatory funnel and allowed to separate for 24 h. The dichloromethane layer containing the PDMS-magnetite complex was collected. The dichloromethane layer containing the PDMS-magnetite complex was dried with magnesium sulfate, vacuum filtered, and concentrated under vacuum. The recovered PDMS-magnetite nanoparticle fluid was washed 3X with methanol (15 mL) and dried under vacuum overnight at 80 °C. TGA was used to determine the composition of the PDMS-magnetite nanoparticle fluid.

5.2.6 Isolation/purification of the one-part PDMS-magnetite nanoparticle fluids through magnetic separations

Two concentrations of the PDMS-magnetite nanoparticle fluids in chloroform (10 mg mL⁻¹ and 1 mg mL⁻¹) were investigated in the magnetic separation studies. The dilute dispersions of the PDMS-magnetite complexes were passed through five magnetic separation columns. Aliquots of the PDMS-magnetite complexes were collected after the 1st and 5th magnetic separations and dried under vacuum at 80 °C overnight. After each magnetic separation of a PDMS-magnetite complex, the columns were washed with chloroform (~20 mL) in the absence of a magnetic field to recover the material removed from the bulk sample. The 1st separation, 5th separation and column-extracted materials were characterized by DLS, TEM, and TGA.

5.2.7 Formation of one-part PDMS-magnetite nanoparticle fluid for determination of radius of gyration using small angle neutron scattering (SANS)

A dispersion of a 3,100 g mol⁻¹ PDMS-magnetite complex in dichloromethane was diluted with additional dichloromethane to a concentration of 2.0 x 10⁻³ g mL⁻¹. The diluted dispersion containing the PDMS-magnetite complex was passed through a magnetic separation column, and
the collected fraction was dried with magnesium sulfate, vacuum-filtered, and then concentrated under vacuum. The recovered PDMS-magnetite nanoparticle fluid was washed 3X with methanol (15 mL each) and dried under vacuum overnight at 80 °C. Particle sizes and size distributions of the PDMS-magnetite ferrofluid were examined using TEM.

5.2.8 Characterization

Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styrigel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

TGA was carried out on the tricarboxylate-functional PDMS and PDMS-stabilized magnetite nanoparticles using a TA Instruments TGA Q500. Samples were first held at 110 °C for 10 min to remove any residual solvent. Samples were then equilibrated at 30 °C and the temperature was ramped at 10 °C min⁻¹ to 700 °C in a nitrogen atmosphere. Char yields were recorded at the maximum temperature. TGA was used to determine the composition of the PDMS-magnetite nanoparticle fluids.

TEM was conducted using a Philips EM-420 field-emission-gun transmission electron microscope. Samples of the polymer-magnetite complexes were dispersed in water through probe sonication and analyzed after being cast onto amorphous carbon-coated copper grids. The eucentric height and focus were set consistently for each sample. The microscope was equipped with a 2000 x 3000 pixel digital imaging system, and images were acquired at a magnification of 96 kx, corresponding to a resolution of 3.7 pixels nm⁻¹. At least 2000 particles taken from five
separate images were used for image analysis. Particle distribution analyses were performed using Reindeer Graphics’ Fovea Pro 4 plug-in for Adobe Photoshop 7.0.

A Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) was used to conduct dynamic light scattering (DLS) experiments. A 4.0 mW, solid-state He-Ne laser at a wavelength of 633 nm was the incident light source. The NanoZS measures at a scattering angle of 173°, which reduces the effects of multiple scattering and contaminants such as dust. Malvern’s Zetasizer Nano 4.2 software was used to calculate intensity, volume and number average diameters utilizing an algorithm that transforms time-varying intensities to particle diameters.128

Magnetic properties were determined using a 7T MPMS Squid magnetometer from Quantum Design. Hysteresis loops were measured at 300K and 5K. The magnetite specific magnetization values were obtained by chemically determining the Fe concentration of the samples following acid digestion using ICP-AES. These values agreed well with the values obtained from TGA.

5.2.9 Small angle neutron scattering
SANS measurements were made on the NG3-SANS beamline at the NIST Center for Neutron Research at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD. Three sample-to-detector distances were used (1.3, 4 and 13.2 m) to cover a q-range from 0.0026-0.33 Å⁻¹ using a mean neutron wavelength of 6 Å. All patterns were collected at 35 °C. Data reduction and preliminary analysis were carried out using IGOR Pro 6 and macros provided by NIST. The data were normalized to a standard poly(methyl methacrylate) sample.

SANS patterns were collected on three dilute solutions of a PDMS ferrofluid and one pattern on a neat one-part ferrofluid. Each of the dilute samples had 1.5 wt% of the PDMS
ferrofluid dispersed in a carrier fluid. The carrier fluids were fully deuterated THF, which is a
good solvent for PDMS, fully deuterated acetone:toluene (6:5 volume ratio), which is a theta
solvent for PDMS, and a non-functional 6,000 g mol\(^{-1}\) PDMS oligomer. Samples were loaded
into demountable titanium sample cells with quartz windows. The cells were cleaned thoroughly
prior to use.

5.2.10 Density distribution model to predict sizes of the PDMS-magnetite
nanoparticle complexes

Modeling of the PDMS-magnetite complexes to predict their sizes in various solutions was
based on methods developed by Mefford et al. and Zhang et al.\(^{7,9,10}\) TEM was used to image the
particles (only the magnetite component of the complexes is visualized), and the size distribution
of the magnetite was fitted with a Weibull probability distribution \(P(r)\) as shown in equation
5.1.

\[
P(r) = \frac{c}{b} \left( \frac{r}{b} \right)^{c-1} \exp \left[ - \left( \frac{r}{b} \right)^c \right]
\]

Eq. 5.1

Here, \(r\) is the particle radius and \(b\) and \(c\) are the Weibull shape and scale parameters, respectively.

The average surface area of the magnetite was calculated from the particle size distribution
derived from TEM. Combining the average surface area with the average polymer loading per
mass of complex (from TGA) gives an average number of chains per magnetite surface area, \(\alpha\), as
shown in equation 5.2.

\[
\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag}}{3 M_n W_{mag}} \int_0^\infty r^3 P(r) dr
\]

Eq. 5.2

\[
\int_0^\infty r^2 P(r) dr
\]

Here, \(\rho_{mag}\) is the density of magnetite (5.21 g cm\(^{-3}\)),\(^{130}\) \(W_{mag}\) is the weight fraction of the complex
that is magnetite, \(N_{Av}\) is Avogadro’s number, and \(M_n\) is the polymer number average molecular
weight. Through application of a modified version of the Vagberg density distribution model, the average radius of the PDMS-magnetite complexes was calculated as

\[ R_m(r) = \left( \frac{8N_k f(r)^{1-\nu} L_k \nu}{4^{1-\nu/3} \nu^{1/2}} + r^{1/\nu} \right)^{\nu} \]  

Eq. 5.3

where \( N_k \) is the number of Kuhn segments, \( L_k \) is the Kuhn length, \( \nu \) is the Flory exponent, and \( f(r) \) is the number of chains per particle, which were calculated from equation Z.4.

\[ f(r) = 4\pi r^2 \alpha \]  

Eq. 5.4

Different modes of the complex distribution were calculated through a Weibull probability distribution fit (Equations 5.5, 5.6, and 5.7) where \( d_n \), \( d_v \), and \( d_i \) are the number, volume, and intensity average diameters, respectively.

\[ d_n = 2 \int_0^\infty R_m(r)P(r)dr \]  

Eq. 5.5

\[ d_v = 2 \int_0^\infty R_m(r)^4 P(r)dr \]  

Eq. 5.6

\[ d_i = 2 \int_0^\infty R_m(r)^6 P(r)dr \]  

Eq. 5.7

### 5.3 Results and Discussion

#### 5.3.1 Synthesis of PDMS-magnetite nanoparticle fluids without solvent

The synthetic procedure for obtaining a tricarboxylate-functional PDMS oligomer has been previously reported (Figure 5.1). Great care was taken to purify all solvents and reagents for the D₃ polymerizations to prevent premature termination of the growing PDMS chains. The living anionic polymerization of D₃ yielded polymers with good control over molecular weights and narrow molecular weight distributions, as confirmed by NMR and GPC (Table 5.1). A series
of trivinylsilyl-terminated PDMS oligomers were synthesized with targeted molecular weights ranging from 3,000 to 10,000 g mol\(^{-1}\). The molecular weights obtained from GPC and NMR were in close agreement with the targeted values.

**Figure 5.1.** Synthesis of tricarboxylate-functional PDMS oligomer for stabilizing fluids and dispersions of magnetite nanoparticles.

**Table 5.1.** Trivinyl-terminated PDMS molecular weights and distributions.

<table>
<thead>
<tr>
<th>Targeted Trivinyl-PDMS MW</th>
<th>(M_n) (g mol(^{-1}))</th>
<th>(\text{PDI})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(^1\text{H NMR})</td>
<td>GPC</td>
</tr>
<tr>
<td>3,000</td>
<td>3,050</td>
<td>3,150</td>
</tr>
<tr>
<td>5,000</td>
<td>5,100</td>
<td>4,830</td>
</tr>
<tr>
<td>7,000</td>
<td>6,900</td>
<td>6,910</td>
</tr>
<tr>
<td>10,000</td>
<td>10,200</td>
<td>9,850</td>
</tr>
</tbody>
</table>
Tricarboxylate-functional PDMS magnetite dispersion stabilizers were synthesized via the ene-thiol addition of mercatoacetic acid to the trivinylsilyl-terminated PDMS series. Complete conversion of the vinyl groups was promoted by thoroughly deoxygenating the reaction mixtures before the additions. Excess mercaptoacetic acid was removed from the polymer during isolation by precipitating the polymer into a methanol/water mixture, yielding well-defined tricarboxylate-functional PDMS oligomers. The addition of mercaptoacetic acid across the vinylsilane groups was confirmed by $^1$H NMR (Figure 5.2).

**Figure 5.2.** $^1$H NMR confirms the addition of mercaptoacetic acid across vinylsilane groups on a 3,100 g mol⁻¹ trivinyl-terminated PDMS. Upper spectrum is trivinylsilyl-terminated PDMS; lower spectrum is the tricarbonylate-functional PDMS.

Previous reports have described ferrofluids comprised of PDMS-magnetite nanoparticle complexes dispersed in PDMS carrier fluids.⁴⁻⁹,¹⁰ However, concern for the long-term stability of these magnetic nanoparticle dispersions has led to research efforts to improve the design of these hydrophobic magnetic fluids. Advancements in the synthesis and purification of the PDMS-
magnetite complexes have allowed for the formation of "one-part" fluids, where the coated magnetite complexes make up the fluids, without adding any non-functional PDMS oligomer as the carrier. These PDMS-magnetite nanoparticle complexes were prepared by adsorbing the tricarboxylate-functional PDMS onto cationic magnetite surfaces in a high-shear interfacial process. The shear forces helped to decrease the particle sizes of the complexes during the magnetite co-precipitation and subsequent polymer adsorption steps. Magnetic field-induced separations were employed to purify the PDMS-magnetite complexes to narrow the particle size distributions. After the separation(s), neat one-part PDMS-magnetite nanoparticle fluids were obtained with compositions close to those targeted (approximately 30 wt% magnetite and 70 wt% PDMS coating) as determined by TGA.

5.3.2 Magnetic separation study of one-part PDMS-magnetite nanoparticle fluids

Mefford et al. described the use of magnetic separation techniques to narrow the particle size distribution of a 3,000 g mole\(^{-1}\) PDMS-magnetite complex.\(^9\) We now report the synthesis and characterization of a series of fluids prepared with different molecular weight oligomers adsorbed on the magnetite surfaces. Additionally, the effect of molecular weight of the PDMS coating on the magnetic separation behavior of PDMS-magnetite complexes with similar compositions was investigated.

Table 5.2 shows the experimental compositions and sizes (by dynamic light scattering) for the series of magnetic fluids containing different molecular weight PDMS coatings before any magnetic separation. Size predictions made with a density distribution model were compared to experimental DLS data to garner information about the colloidal stabilities of the complexes. The sizes measured by DLS were significantly larger than those predicted from the model. This is
attributed to slight particle aggregation that is inherent in the process utilized for forming the magnetite particles.

Removal of the slightly-aggregated complexes from the total sample was accomplished through repeated magnetic separations. Table 5.2 also shows DLS number average diameters compared to those predicted by the model after five magnetic separations using a complex concentration of 1 mg mL$^{-1}$ in chloroform. The model matches the DLS results significantly better (average of 16% deviation from the model) following the 5th magnetic separation, indicating that indeed the larger aggregates can be removed from the solution by this method.

Table 5.2. PDMS-magnetite complex compositions and sizes before any magnetic separations and after five magnetic separations (1 mg mL$^{-1}$ separation concentration). Five magnetic separations remove larger aggregates and the resulting complex sizes agree with the density distribution model better.

<table>
<thead>
<tr>
<th>PDMS-Magnetite Complex</th>
<th>% Magnetite</th>
<th>0 Separations</th>
<th>5th Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DLS $D_n$ (nm)$^*$</td>
<td>Model $D_n$ (nm)</td>
</tr>
<tr>
<td>3.050 g mol$^{-1}$</td>
<td>35</td>
<td>29.6</td>
<td>18.1</td>
</tr>
<tr>
<td>5.100 g mol$^{-1}$</td>
<td>29</td>
<td>31.0</td>
<td>22.5</td>
</tr>
<tr>
<td>6.900 g mol$^{-1}$</td>
<td>24</td>
<td>30.1</td>
<td>26.6</td>
</tr>
<tr>
<td>10.200 g mol$^{-1}$</td>
<td>26</td>
<td>48.4</td>
<td>29.7</td>
</tr>
</tbody>
</table>

*D$_n$ = Number Average Diameter

The separation columns were comprised of iron granules tightly packed into 3-mL syringes. The syringes were 6.60 cm in length with an inner diameter of 1.00 cm. The length of the iron granules in the separation columns was approximately 2.45 cm with a diameter of 1.00 cm. Four doughnut-shaped magnets were placed around the circumferences of the iron granule-packed syringes to form the magnetic separation columns (Figure 5.3), and a hall probe was used to measure the magnetic field generated by the separation columns. Great care was taken at each step of the column formation to ensure reproducibility.
Figure 5.3. Separation columns were comprised of iron granules packed into a 3 mL syringe using glass wool (left). Doughnut-shaped magnets were placed around the circumference of the column to perform the magnetic separations of the dilute PDMS-magnetite complexes (right).

Initial experiments investigated the magnetic separation of PDMS-magnetite complexes dispersed in chloroform at a concentration of 10 mg mL\(^{-1}\). DLS was used to compare the particle size distributions of the complexes before magnetic separation and after the 1\(^{st}\) and 5\(^{th}\) separations. Similar magnetic separation profiles were observed for the 5,100 and 6,900 g mole\(^{-1}\) PDMS-magnetite complexes (Figure 5.4). As the number of magnetic separations was increased, the particle size distributions became narrower.
Different features were observed in the separation profile of the 10,200 g mol$^{-1}$ PDMS-magnetite complex (Figure 5.5). A significant amount of the larger particles were removed in the 1$^{st}$ separation of the complex, followed by no appreciable narrowing of the particle size distribution between the 1$^{st}$ and 5$^{th}$ magnetic separations. The particle size distribution of the 10,200 g mol$^{-1}$ PDMS-magnetite complex after five separations was broader than for the ferrofluids having the lower molecular weight PDMS oligomers.
DLS analysis of the materials retained in the separation columns did not reveal any conclusive trend based on molecular weight of the PDMS stabilizer. Similar size materials were removed during each magnetic separation of the 5,100 and 6,900 g mol⁻¹ PDMS-magnetite complexes (Table 5.3). It appears that stabilizer molecular weight does not affect the size of particles fractionated out of the bulk complexes during the magnetic separations.

Table 5.3. Comparable sizes in chloroform were observed in the DLS analysis of the separation and column-extracted materials for the 5,100 g mol⁻¹ and 6,900 g mol⁻¹ PDMS-magnetite complexes.
To eliminate the possibility of saturation in the magnetic separation columns, the concentration of PDMS-magnetite complexes in chloroform was reduced to 1 mg mL$^{-1}$. These separation experiments were performed in the dilute colloidal regime. The mass of the column-extracted materials was measured after each magnetic separation and the separation profiles of the PDMS-magnetite complexes were plotted (Figure 5.6). Approximately 80% of the total mass was removed after five separations, regardless of the molecular weight of the PDMS stabilizer.

![Figure 5.6](image)

**Figure 5.6.** Total mass removed as a function of PDMS stabilizer molecular weight and number of magnetic separations.

TGA data of the 5,100 and 6,900 g mol$^{-1}$ PDMS-magnetite complexes collected during magnetic separations is shown in Figure 5.7. A decrease in char yield was observed between the 1$^{st}$ and 5$^{th}$ magnetic separations for both samples, indicating an increase in polymer content in the
PDMS-magnetite complexes. This trend suggests that larger magnetite particles with less adsorbed polymer were being fractionated out of the bulk PDMS-magnetite complexes during the magnetic separations. Furthermore, this fractionation of the larger materials from the heterogeneous PDMS-magnetite complexes appears to not be influenced significantly by the molecular weight of the tricarboxylate-functional PDMS dispersion stabilizer.

**Figure 5.7.** Polymer content of the PDMS-magnetite complexes increases with the number of magnetic separations.

Based on the finding that larger particles with less adsorbed polymer were being extracted during separations, the materials that were extracted in the 1st column were analyzed via TGA (Figure 5.8). As expected, higher char yields were observed for these extracted materials due to a
significantly lower polymer content bound to the particles. Magnetic separations of these ‘one-part’ PDMS ferrofluids appear to be influenced more by the heterogeneity of particles sizes than the PDMS stabilizer molecular weight.

Figure 5.8. The column-extracted materials during the magnetic separation of the PDMS-magnetite complexes have higher magnetite contents than the starting materials.

Due to the sensitivity of PDMS chains to degradation in the presence of acids, TGA was utilized to investigate the thermal stabilities of the 3,050 g mol\(^{-1}\) tricarboxylate-functional PDMS stabilizer and the 3,050 g mole\(^{-1}\) PDMS-magnetite complex before magnetic separation (Figure 5.9). The onset of degradation for both materials was approximately 300 °C, and thus it was concluded that these polymers and complexes had sufficient thermal stability against chemical
degradation to be utilized in biotechnological applications at 37 °C. The PDMS stabilizer in a N₂ atmosphere showed ~100% weight loss in the TGA by 700 °C, indicating that char yield data obtained from the PDMS-magnetite complexes can be attributed to the magnetite composition.

Figure 5.9. A similar "onset-of-degradation temperature" was observed in the TGA analysis of the tricarboxylate-functional PDMS and PDMS-magnetite complexes.

5.3.3 Formation of one-part PDMS-magnetite nanoparticle fluid for SANS characterization

A one-part 3,100 g mol⁻¹ PDMS-magnetite nanoparticle fluid was investigated by SANS to measure the radius of gyration (R_g) of the complex. This experimental measurement was compared to the predicted R_g derived from the density distribution model. After one magnetic separation, this fluid had a composition of 37 wt% magnetite and 63 wt% PDMS as determined
by TGA analysis. Additionally, a non-functional PDMS was utilized as a carrier solvent for the fluid and compared in the SANS measurements. Table 5.4 contains the molecular weight data for the 3,100 g mol⁻¹ PDMS dispersion stabilizer and the non-functional PDMS carrier.

Table 5.4. PDMS molecular weights and distributions.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Mn (g mol⁻¹)</th>
<th>¹H NMR</th>
<th>GPC</th>
<th>PDI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS-trivinyl</td>
<td>3,100</td>
<td>2,900</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>PDMS</td>
<td>5,800</td>
<td>6,300</td>
<td>1.03</td>
<td></td>
</tr>
</tbody>
</table>

*PDI = Polydispersity Index

The one-part 3,100 g mol⁻¹ PDMS-magnetite complex after one magnetic separation was characterized by TEM. Figure 5.10 shows a representative TEM image and the particle histogram obtained from image analysis with number weighted and volume weighted average radii of 3.17 nm and 4.35 nm, respectively.

Figure 5.10. A particle size distribution fitted with a Weibull probability distribution (left) and a representative TEM image (right) for the 3,100 g mol⁻¹ PDMS-magnetite complex after one magnetic separation.
5.3.4 Magnetic properties of the PDMS-magnetite nanoparticle complex

Hysteresis loops of the 3,100 g mole\(^{-1}\) PDMS-magnetite complex were measured at 300 K and at 5 K (Figure 5.11). The saturation magnetization of the one-part ferrofluid is 23.8 emu g\(^{-1}\), which corresponds to a magnetite specific saturation magnetization of 76.5 emu g\(^{-1}\). The low field volume susceptibility of the ferrofluid at 300 K in SI units was 0.45. Based on these results, the magnetite nanoparticles are superparamagnetic (i.e. no hysteresis).

![Hysteresis loop of the one-part 3,100 g mol-1 PDMS-magnetite ferrofluid at 300 K and 5 K. Insert is the low field behaviour at the same temperatures.](image)

**Figure 5.11.** Hysteresis loop of the one-part 3,100 g mol-1 PDMS-magnetite ferrofluid at 300 K and 5 K. Insert is the low field behaviour at the same temperatures.

5.3.5 Comparison of predicted and experimental sizes of the complexes

Calculations of sizes of the 3,100 g mole\(^{-1}\) PDMS-magnetite complexes in solution and their interparticle forces utilizing DLVO theory and a density distribution model, together with experimental measurements of these parameters, help us relate the molecular compositions to extension of the polymer brushes from the magnetite surfaces and colloidal stabilities. **Table 5.5** shows a comparison of sizes predicted by the model and those measured by DLS in a good
solvent (THF) and a theta solvent (6:5 v:v acetone:toluene). Measurements were also made in the corresponding deuterated solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Density Distribution Model</th>
<th>Dynamic Light Scattering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_i$ (nm)</td>
<td>$D_v$ (nm)</td>
</tr>
<tr>
<td>THF</td>
<td>20.9</td>
<td>19.8</td>
</tr>
<tr>
<td>D-THF</td>
<td>20.9</td>
<td>19.6</td>
</tr>
<tr>
<td>6:5 Acetone:Toluene</td>
<td>19.8</td>
<td>18.7</td>
</tr>
<tr>
<td>6:5 D-Acetone:D-Toluene</td>
<td>19.8</td>
<td>18.7</td>
</tr>
</tbody>
</table>

In all cases, the DLS data shows significantly larger complexes than those predicted from the model. As described in equation 5.5, 5.6, and 5.7, the intensity, volume, and number average diameters differ because they are weighted differently. The intensity average diameter is weighted heavily towards larger particles (particle radius weighted as $r^6$), followed by the volume average diameter (weighted as $r^3$) which is less weighted towards larger particles than the intensity average diameter but more so than the number average diameter, and then the number average diameter, which just scales with the radius of the complex. Large deviations were observed for all three distributions, with the deviation decreasing as the weighting of the particles size decreased. This suggests that the particle size distribution that was used for the core may be too narrow or that the particles may be clustering because the intensity averages are most affected by larger particles. The DLS measurements are sensitive to these larger particles, whereas the model—which assumes ideal conditions (i.e. no clustering, dilute solution)—is not.
An increase in all three averages of the diameters was observed for measurements made in the good solvent when compared to those made in the theta solvent. This makes intuitive sense because a polymer brush should extend further from the surface in a good solvent compared to a theta solvent. However, this also tells us that if clustering is indeed causing the deviations shown in Table 5.6, the clustering is occurring before adsorption of the PDMS instead of due to pair-pair interactions between two PDMS-magnetite complexes. This is because a more extended brush in a good solvent should provide a higher degree of steric repulsion than in a theta solvent and so the diameters in the good solvent would be smaller if the clustering was due to pair-pair interactions.

**Table 5.6.** Comparison of a bulk volume fraction calculation to that obtained from the density distribution model for the 3,100 g mol⁻¹ PDMS-magnetite complex.

<table>
<thead>
<tr>
<th>% Magnetite</th>
<th>Φ&lt;sub&gt;PDMS&lt;/sub&gt;(bulk)</th>
<th>Φ&lt;sub&gt;PDMS&lt;/sub&gt;(Model)</th>
<th>Deviation Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.37</td>
<td>0.65</td>
<td>0.21</td>
<td>3.1</td>
</tr>
<tr>
<td>0.40</td>
<td>0.61</td>
<td>0.20</td>
<td>3.1</td>
</tr>
<tr>
<td>0.50</td>
<td>0.49</td>
<td>0.16</td>
<td>3.1</td>
</tr>
<tr>
<td>0.60</td>
<td>0.40</td>
<td>0.13</td>
<td>3.1</td>
</tr>
<tr>
<td>0.70</td>
<td>0.32</td>
<td>0.10</td>
<td>3.2</td>
</tr>
</tbody>
</table>

5.3.6 Radius of gyration (R<sub>g</sub>) of the 3,100 g mol⁻¹ PDMS-magnetite complex in solution

**Figure 5.12** shows the SANS pattern from dilute solutions of the 3,100 g mole⁻¹ PDMS ferrofluid in both the good and theta solvents. This data was fitted with a Guinier curve at low q (inset Figure 5) to determine the radius of gyration (R<sub>g</sub>) of the PDMS coated particles in solution. Due to the high relative contrast between the PDMS and the deuterated solvent, the value of R<sub>g</sub>
from the fit is dominated by the PDMS density distribution around the magnetite particle. The values for $R_g$ are listed in Table 5.7.

![Figure 5.12](image)

**Figure 5.12.** SANS pattern from PDMS coated magnetite nanoparticles in deuterated tetrahydrofuran (D-THF) and deuterated acetone/toluene, which are good and theta solvents for PDMS respectively. Insert is the Guiner fit to the data used to determine the radius of gyration ($R_g$).

**Table 5.7.** Comparison of $R_g$ from SANS to those calculated from the density distribution model for the 3,100 g mol$^{-1}$ PDMS-magnetite complex.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$R_g$ (SANS) (Å)</th>
<th>$R_g$ (Bulk) (Å)</th>
<th>$R_g$ (Model) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-THF</td>
<td>132.6</td>
<td>82.6</td>
<td>82.6</td>
</tr>
<tr>
<td>6:5 D-Acetone:D-Toluene</td>
<td>135.3</td>
<td>80.2</td>
<td>80.2</td>
</tr>
</tbody>
</table>
One can calculate the radius of gyration from the Density Distribution model to compare to the \( R_g \) value obtained from SANS by integrating the second moment of the scattering length density (SLD) profile as shown in equation 5.8.

\[
< R_g^2 >= \frac{\int_0^R (SLD_{\text{core}} - SLD_{\text{solvent}}) h^4 \, da}{\int_0^R (SLD_{\text{core}} - SLD_{\text{solvent}}) h^2 \, da} + \frac{\int_0^R (\phi_{\text{PDMS}} SLD_{\text{PDMS}} - \phi_{\text{PDMS}} SLD_{\text{solvent}}) h^4 \, da}{\int_0^R (\phi_{\text{PDMS}} SLD_{\text{PDMS}} - \phi_{\text{PDMS}} SLD_{\text{solvent}}) h^2 \, da}
\]

Eq. 5.8

Here, \( h \) is the distance from the center of the particle extending out in the radial direction, \( R_c \) is the volume average radius of the magnetite core, \( R_v \) is the volume average radius of the complex, SLD is the scattering length density which for the magnetite core is 7.07, for the PDMS is 0.07 and for the solvent is 6.35 for d-THF and 5.51 for the 6:5 d-acetone:d-toluene mixture. For \( h < R_c \) (volume weighted average), the volume fraction of solvent is zero and the volume fraction of the core is constant. For \( R_c < h < R_v = d_v/2 \), the volume fraction of the polymer as a function of distance from the central core can be calculated from the Vagberg model. Vagberg defines the blob diameter and the number of monomers per blob as shown in Equations 5.9 and 5.10, respectively.

\[
\xi(h) = \frac{4h}{f^{1/2}} \quad \text{Eq. 5.9}
\]

\[
\text{monomers per blob} = \left(\frac{\xi(h)}{a_s}\right)^{1/v} \quad \text{Eq. 5.10}
\]

\( \xi(h) \) is the blob diameter and \( a_s \) is the Kuhn segment length. The volume of monomer in a blob can then be calculated as shown in Equation 5.11.

\[
V_{\text{monomer}} = \frac{m_s}{\rho_s N_A} \frac{\text{monomers per blob}}{\text{blob}} = \frac{m_s}{\rho_s N_A} \left(\frac{4h}{a_s f^{1/2}}\right)^{1/v}
\]

Eq. 5.11
Here, \( m_s \) is the molecular weight of the monomer, \( \rho_s \) is the density of the polymer, and \( N_A \) is Avagadro’s number. The volume of the blob can then be calculated as shown in Equation 5.12.

\[
V_{\text{blob}} = \frac{4}{3} \pi \xi(h)^3 \tag{Eq. 5.12}
\]

The volume fraction of PDMS in the extended brush as a function of the distance from the center of the complex can thus be calculated as shown in Equation 5.13.

\[
\phi_{\text{PDMS}}(h) = \frac{V_{\text{monomer}}}{V_{\text{blob}}} = \frac{m_s}{\rho_s N_A} \left( \frac{4h}{a_s f^{1/2}} \right)^{1/\nu} \frac{4}{3} \pi \left( \frac{4h}{f^{1/2}} \right)^3 \tag{Eq. 5.13}
\]

Thus, the volume fraction of the solvent in the PDMS brush is \((1 - \Phi_{\text{solvent}})\).

5.3.7 Comparison of the \( R_g \) from SANS to the Density Distribution model calculation

The radii of gyration \((R_g)\) were obtained from SANS and also calculated using the density distribution model as described earlier. This is based on the scattering length density (SLD) profiles changing with increased distance from the magnetite surface due to increased solvation of the polymer. Thus, the model must be used to predict the volume fraction profile of the polymer as a function of distance from the surface to accurately predict the \( R_g \).

To validate the volume fraction profile derived from the density distribution model, it was compared to the known average bulk volume fraction of polymer in the corona shell for a single complex. This was done by treating the magnetite-PDMS complex as a solid magnetite core surrounded by a shell consisting of a polymer-solvent mixture. Because the average diameter of the complex is known (calculated from the density distribution model), the average diameter of the core is also known (from TEM), and the polymer to magnetite ratio is known (from TGA), the volume fraction can be calculated as shown in Equation 5.14.
Here, $m_{\text{poly}}$ is the mass of polymer per complex, $\rho_{\text{poly}}$ is the bulk density of the polymer, and $a$ is the distance from the magnetite surface to the outer edge of the complex.

An average bulk volume fraction can then be calculated from the density distribution model by applying a common averaging technique as shown in Equation 5.15.

\[
\langle \phi_{\text{PDMS}} \rangle = \frac{\int_0^{2\pi R_g} \int_0^{2\pi R_s} \phi_{\text{PDMS}}(h) dh d\theta}{\int_0^{2\pi R_s} dh d\theta}
\]

As Table 5.6 shows, there is a consistent deviation of the density distribution model from the bulk volume fraction calculation (bulk calculation is 3X the model calculation). Recall that the DLS results (Table 5.6) indicate some clustering of the magnetite cores. This would significantly affect interpretation of the TGA results, namely that the magnetite core may not be a fixed size but some may be multiple magnetite particles coated with one polymer brush layer. Thus, the calculation of the volume of the polymer-solvent shell may be erroneous because it depends on a single magnetite particle with a radius of ~3.2 nm.

So the important question is whether this difference in volume fraction affects the radius of gyration calculation determined from the scattering length densities. Obviously, this will have some effect, but whether that effect is significant is what is important. Table 5.7 shows the comparison of the $R_g$ from SANS to those calculated from the density distribution model using both the bulk and model calculated volume fractions.

The first observation is that neither of the calculated radii of gyration are close to the values obtained by SANS. The second, and perhaps more important, observation is that even with

\[
\tilde{\phi}_{\text{bulk}} = \frac{m_{\text{poly}}}{\rho_{\text{poly}}} \frac{3}{4\pi a^3}\]

\text{Eq. 5.14}
the differences in volume fraction, the calculated radii of gyration are remarkably close. This indicates that the bulk volume fraction of polymer to solvent is not a vitally important parameter when determining the radius of gyration. Additionally, this is consistent with the DLS results, which indicate that the radius of gyration measured by SANS was not of a complex consisting of an individual core and polymer brush, but some mixture of individual and clustered cores coated with a PDMS brush. Further experiments could be done on PDMS ferrofluids passed through a magnetic separation column five times and then comparison of the density distribution model for both size and radius of gyration would be more definitive.

5.3.8 Scattering from one-part 3,100 g mol\(^{-1}\) PDMS-magnetite nanoparticle ferrofluid

For dilute particle fluids (i.e. when the volume fraction of scattering objects is less than 1 to 5\%), the SANS scattering patterns scale perfectly with the concentration of particles. However, when the sample is concentrated, scattering caused by the spacing of objects becomes significant. In modeling, this interparticle scattering is accounted for by the structure factor \((S(q))\), so that the intensity of the scattering \(I(q)\) is given by

\[
I(q) = \phi F(q) S(q)
\]

where \(\phi\) is the volume fraction, \(F(q)\) is the form factor which describes the scattering from a single particle and \(S(q)\) is the structure factor that describes the scattering between particles. The structure factor, \(S(q)\), can be experimentally determined by comparing the scattering from a concentrated sample to that from a dilute sample in which \(S(q)\) approaches 1. \(S(q)\) of the concentrated sample, \(S(q)_{\text{conc}}\), is given by

\[
S(q)_{\text{conc}} = \frac{\phi_{\text{dil}} I(q)_{\text{conc}}}{\phi_{\text{conc}} I(q)_{\text{dil}}}
\]

where \(\phi_{\text{dil}}\) and \(\phi_{\text{conc}}\) are the volume fractions of the scattering objects in the dilute and concentrated samples and \(I(q)_{\text{conc}}\) and \(I(q)_{\text{dil}}\) are the scattering intensities for the dilute and
concentrated samples, provided that $F(q)$ is the same for both samples and that $S(q)$ for the dilute sample approaches unity.

To determine the $S(q)$ for the one-part ferrofluid, we measured the scattering intensity of both the one-part ferrofluid and a sample of the ferrofluid diluted with a non-functional PDMS oligomer. In the one-part ferrofluid, the scattering was from the magnetite cores relative to the PDMS coating and the cores had a volume fraction of 9.81%. Because we used the PDMS oligomer as the carrier fluid in the dilute sample, the scattering was still only from the magnetite cores, i.e. $F(q)$ was the same for both samples, and the volume fraction of the magnetite cores was 0.14%. The scattering patterns along with the $S(q)$ calculated using Equation 5.17 are shown in Figure 5.13.

![Figure 5.13](image.png)

**Figure 5.13.** SANS pattern for concentrated ferrofluid and for ferrofluid diluted with PDMS. The line is the concentration scaled scattering pattern of the dilute ferrofluid. $S(q)_{conc}$ is the experimentally determined $S(q)$ of the concentrate ferrofluid from Equation 5.17.
The first peak in the S(q) corresponds to the nearest neighbor center-to-center distance of the magnetite cores (171 Å). If we assume that the number of chains per unit surface area (\( \alpha \)) is constant for all particles and that the particles are randomly loose packed with a packing fraction of 0.625, then it is possible to calculate the volume weighted average spacing between magnetite centers. This value is 178 Å in good agreement with the measured values for S(q).

5.4 Conclusions
In this paper, a series of ‘neat’ one-part PDMS-magnetite nanoparticle fluids were synthesized. The polymer:magnetite composition of the fluids were similar (~ 30 wt% PDMS:70 wt% magnetite), however the molecular weight of the PDMS dispersion stabilizer was varied (3,000-10,000 g mol\(^{-1}\)). The effect of magnetic separation was studied by passing dilute solutions of the fluids in chloroform through columns consisting of magnetized iron filings. DLS characterization revealed that larger particles of similar size were fractionated out of the separated materials independent of tricarboxylate-functional PDMS molecular weight. As particle size distributions of the PDMS-magnetite complexes narrowed with subsequent magnetic separations, the experimental DLS complex sizes were closer to the predicted complex sizes from the density distribution model.

5.5 Acknowledgements
The authors are grateful for the financial support of the NSF/ARC Materials World Network for the Study of Macromolecular Ferrofluids (DMR-0602932 and LX0668968), and to NIST Center for Neutron Research at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD
CHAPTER 6: Functional Polydimethylsiloxanes and Poly(dimethylsiloxane-b-acrylate) Block Copolymers via Living Polymerizations

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6.1 Introduction

Polydimethylsiloxane (PDMS) oligomers with terminal functionality have been of interest for many years, primarily due to their capacity to be incorporated through functional endgroups into block copolymers and networks\textsuperscript{166-168}. The flexible nature of the Si-O units in the backbone produces materials with very low glass transition temperatures ($\sim -123^\circ$C for polydimethylsiloxane) and the Si-O bond is unusually thermally stable at elevated temperatures. This gives rise to hydrophobic elastomer materials with a wide temperature use range since the materials can maintain flexibility at very low temperatures and resist thermo-oxidative degradation at elevated temperatures for extended periods (to $\sim 200^\circ$C). Moreover, the hydrophobic PDMS tends to nanophase separate from organic components in block copolymer architectures to produce multi-phase materials even at fairly low block lengths\textsuperscript{166-168}. The hydrophobic-hydrophilic nature of the PDMS-b-polyacrylate copolymers described herein are interesting because they can form multi-phase materials that may be useful in a variety of biomedical technologies due to interactions of the polyacrylate phase with water.

PDMS oligomers with functional groups on each end (difunctional PDMS) are usually
prepared in so-called redistribution or equilibration reactions. The polydispersity indices of such oligomers are close to two due to the variety of chain breaking, back-biting and ring-opening reactions that occur in these polymerizations. Due to the nature of the reaction sequences, it is not possible in such reactions to make perfectly monofunctional oligomers. By contrast, monofunctional PDMS is usually made through a living ring-opening reaction of the strained, 6-membered \( \text{D}_3 \) monomer. The reactions are typically initiated with an alkyl lithium to yield a non-functional initiating end, then the reactions are terminated with a functional chlorosilane. Far fewer different types of functional groups have been incorporated onto these monofunctional PDMS oligomers. This paper describes a facile and versatile method of introducing a variety of functional group types on one end only with the potential to form many different monofunctional types of materials. This paper describes the synthesis of an amino-functional material as an example and precursor to the particular block copolymers discussed. The method of functionalization, however, utilizing functional thiol reagents could be utilized to form other types of endgroups.

### 6.2 Experimental

#### 6.2.1 Materials

Hexamethylcyclotrisiloxane (\( \text{D}_3 \), Gelest, Inc., 98%) was dried over calcium hydride and sublimed prior to use. Cyclohexane (Fischer Scientific, HPLC grade) was stirred with concentrated sulfuric acid for 48 h, washed with deionized water until neutral and dried over magnesium sulfate. The cyclohexane was then stirred over calcium hydride, fractionally distilled, stored over sodium in a nitrogen atmosphere, and distilled just prior to use. Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution
reached a deep purple, and fractionally distilled just prior to use. Chloroform (HPLC Grade), dichloromethane (HPLC Grade) and dimethylformamide (DMF, 99.8%) were purchased from EMD Chemicals and used as received. 2,2'-Azobisisobutyronitrile (AIBN, 98%) and n-butyllithium (2.5 M solution in hexanes) were purchased from Aldrich and used as received. Vinylidimethylchlorosilane (Gelest) was used as received. 2-Bromoisobutyril bromide (97%), copper(1) bromide (99.998%), 2-mercaptoethylamine hydrochloride (98+%), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA, 98%) and triethylamine (99+) were purchased from Alfa Aesar and used as received. tert-Butyl acrylate (Alrich, 98%) was distilled to remove hydroquinone monomethyl ether inhibitor prior to use. Toluene (Burdick and Jackson, 99.9%) was used as received. Zinc bromide (Aldrich, 99.999%) was used as received.

6.2.2 Synthesis of monofunctional vinyl-terminated PDMS

The synthesis of a targeted 3,000 g mol⁻¹ dimethylvinyl-terminated PDMS was adapted from a previously described procedure. D₃ (35.0 g, 0.157 mol) was sublimed into a flame-dried roundbottom flask containing a magnetic stir bar and purged with nitrogen. Cyclohexane (35 mL) was added to the flask via syringe and the D₃ monomer was dissolved at room temperature. n-Butyllithium (2.5 M, 4.20 mL, 0.0105 mol) was added to the reaction flask via syringe and the solution was stirred for 1 h, followed by the addition of THF (10 mL) to the solution as a reaction promoter. The living anionic polymerization was monitored using ¹H NMR, and at ~90% conversion of monomer (~18 h), the polymer was terminated with an excess of vinylidimethylchlorosilane (2.15 mL, 0.0157 mol). The solution was stirred overnight and then concentrated under vacuum at 40 °C. The product was dissolved in 200 mL of dichloromethane, washed three times with DI water (100 mL), concentrated under vacuum and precipitated into
methanol (300 mL). The recovered monofunctional dimethylvinyl-terminated PDMS oligomer was dried under vacuum at 80 °C overnight. $^1$H NMR confirmed the expected chemical structure.

6.2.3 Functionalization of monofunctional dimethylvinyl-terminated PDMS with cysteamine hydrochloride

A PDMS oligomer with an amine group on one terminus was prepared via a thiol-ene addition of 2-mercaptopethylamine hydrochloride across the vinylsilane endgroup. The dimethylvinyl-terminated PDMS described above (7.0 g, 0.0023 mol vinyl) was added to a flame-dried roundbottom flask containing a magnetic stir bar and was deoxygenated using a freeze-thaw method. In a separate flame-dried roundbottom flask, 2-mercaptoethylamine hydrochloride (3.96 g, 0.0348 mol) and AIBN (0.030 g, 2 x 10$^{-4}$ mol) were dissolved in deoxygenated DMF (5 mL) and sparged with nitrogen for 0.5 h to remove oxygen. The DMF mixture was added to the flask containing the PDMS. The interfacial reaction mixture was heated at 80 °C with rapid stirring for 3 h. $^1$H NMR was used to observe the complete disappearance of the vinyl proton peaks (~6 ppm), indicating quantitative addition of 2-mercaptoethylamine hydrochloride across the vinyl-terminated PDMS. Triethylamine (~4 mL) was added to the reaction mixture until a pH of ~10 was reached to convert the ammonium chloride endgroups to free amines. The product was dissolved in chloroform (200 mL), washed three times with DI water (100 mL each), and concentrated under vacuum at 40 °C. $^1$H NMR confirmed the expected chemical structure.

6.2.4 Synthesis of bromo-terminated PDMS macroinitiator for ATRP

The reaction of a 3,000 g mol$^{-1}$ monofunctional amine-terminated PDMS with 2-bromoisoobutyryl bromide is provided. Monofunctional amine-terminated PDMS (2.5 g, 8.3 x 10$^{-4}$ mol amine) was dissolved in dichloromethane (200 mL) in a flame-dried roundbottom flask containing a magnetic stir bar and purged with nitrogen. Triethylamine (0.18 mL, 0.0013 mol)
was added to the flask and the reaction mixture was cooled to 0 °C. 2-Bromoisobutyryl bromide (0.12 mL, 9.2 x 10^{-4} mol) was added dropwise into the flask, and the reaction mixture was allowed to warm to room temperature and react for 24 h with stirring. The reaction mixture was passed through a Celite column and washed three times with DI water (100 mL). The dichloromethane was removed under vacuum and the product was dried under vacuum at 60 °C for 12 h. The final product was confirmed using ¹H NMR.

6.2.5 Synthesis of PDMS-b-poly(tert-butyl acrylate) (PDMS-b-PtBA)

The synthesis of a targeted 3,000 g mol⁻¹ PDMS-b-8,000 g mol⁻¹ PtBA via ATRP was achieved using the following procedure. PMDETA (0.14 mL, 6.7 x 10⁻⁴ mol) and tert-butyl acrylate (3.0 mL, 0.020 mol) were dissolved in toluene (2 mL) in a flame-dried roundbottom flask containing a magnetic stir bar and sparged with nitrogen for 2 h to remove oxygen. Copper(I) bromide (0.049 g, 3.3 x 10⁻⁴ mol) was added to the flask, forming a green heterogeneous dispersion. Bromo-terminated PDMS (1.0 g, 3.3 x 10⁻⁴ mol) and toluene (2 mL) were added to a separate flame-dried roundbottom flask and deoxygenated using a freeze-thaw method. The PDMS solution was added to the dispersion and the resulting mixture was allowed to react with stirring at 80 °C for 12 h. The reaction mixture was dissolved in chloroform (200 mL), passed through an alumina column and washed three times with DI water. The product was concentrated under vacuum and dried overnight at 60 °C. The ATPR product was characterized using ¹H NMR and GPC.

6.2.6 Mild deprotection of tert-butyl ester groups in PDMS-b-PtBA copolymer

PDMS-b-poly(acrylic acid) was formed using a mild ZnBr₂ deprotection procedure adapted from Wu et al.¹⁷⁴ PDMS-(tBuAcrylate) (0.44 g, 4.2 x10⁻⁵ mol) and ZnBr₂ (2.5 g, 0.011 mol) were dissolved in dichloromethane (5 mL). The reaction mixture was stirred at room temperature
overnight. DI water (50 mL) was added to the solution and stirred for 4 h, and the product was extracted with three washes of dichloromethane (30 mL each). The collected organic layers were dried with magnesium sulfate and concentrated under vacuum. The PDMS-b-poly(acrylic acid) product was characterized with $^1$H NMR and GPC.

6.2.7 Characterization
Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights. Differential scanning calorimetry (DSC) analysis was performed using a TA Instruments DSC Q1000 equipped with a liquid nitrogen cooling system and a helium flow rate of 50 mL/minute. Samples were cooled to -160°C and isothermed for 5 minutes. The samples were then heated at a rate of 5°C/minute to 130°C. The sample were then cooled back to -160°C at a rate of 10°C/minute, followed by a heating rate of 5°C/minute to 130°C. The second heating cycle was reported for thermal transition values.

6.3 Results and Discussion
A monofunctional vinyl-terminated PDMS with a targeted molecular weight of 3,000 g mol$^{-1}$ was synthesized by initiating with n-butyllithium, then propagating the polymerization through the anionic ring-opening of D$_3$. Reaction progress was monitored using $^1$H NMR by comparing the integration of the growing siloxane backbone peak (0 ppm) to the disappearing cyclic trimer monomer peak (0.15 ppm). At the desired conversion of monomer to polymer, the
anionic PDMS chain ends were terminated with vinyl(dimethylchlorosilane). The number average molecular weight ($M_n$) of the monovinyl-terminated PDMS was determined to be 3,000 g mol$^{-1}$ by $^1$H NMR and 3,200 g mol$^{-1}$ by GPC, with a polydispersity index (PDI) of 1.07. The precise molecular weight control and narrow molecular weight distribution observed is representative of the living anionic polymerization of D$_3$ conducted under mild conditions.

An ene-thiol addition of 2-mercaptoethylamine hydrochloride to the vinyl-terminated PDMS was conducted to introduce ammonium functionality on the vinylsilyl terminus. Reaction mixtures were deoxygenated to ensure quantitative conversion of the vinyl groups. A 1.5 molar excess of thiol and ~10 mol% AIBN, both relative to vinylsilane, were added to DMF and stirred interfacially with the neat vinyl-terminated PDMS at 80 °C. The different phases were stirred vigorously with a mechanical stir bar, creating an emulsion to promote interaction between the thiol radical and the vinylsilane terminus. After thiol addition, triethylamine was added to the reaction mixture to form free amine endgroups on the functionalized PDMS. DMF and 2-mercaptoethylamine hydrochloride were removed after the thiol addition with DI water washes. Figure 6.1 shows the synthesis of the monoamino-terminated PDMS.
Figure 6.1. Synthesis of monoamino-terminated PDMS as a precursor for the ATRP macroinitiator.

$^1$H NMR was used to monitor the ene-thiol addition of 2-mercaptoethylamine hydrochloride to the monovinyl-terminated PDMS. Complete disappearance of the vinyl groups (~6 ppm) was observed, indicating addition of ammonium functionality to the PDMS. Figure 6.2 shows the $^1$H NMR spectrum of the functionalized PDMS product in the free amine form. Quantitative addition of amine functionality was confirmed by comparing the integration of the methylene protons next to the siloxane on the butyl endgroup (~ 0.5 ppm) to the methylene protons of the mercaptoethylamine terminus (2.7 ppm and 3.1 ppm). This comparison shows a 1:1 stoichiometric ratio of endgroups on the monoamino-terminated PDMS.
Figure 6.2. $^1$H NMR illustrates the disappearance of vinyl protons on a 3,000 g mol$^{-1}$ monovinyl-terminated PDMS after addition of cysteamine hydrochloride across the vinylsilane group. This spectrum shows the functionalized PDMS in the free amine form.

A monofunctional PDMS macrorinitiator for ATRP was prepared by reacting monoamino-terminated PDMS with 2-bromoisobutyryl bromide, forming an amide linkage in the bromo-terminated PDMS product (Figure 6.3). An excess of triethylamine to acid bromide was used in the reaction to prevent reaction of the acid by-product with the PDMS backbone.
Figure 6.3. Synthesis of a monobromo-terminated PDMS for use as a macroinitiator in the ATRP addition of tert-butyl acrylate.

$^{1}$H NMR was used to confirm quantitative functionalization of the monoamino-terminated PDMS with the acid bromide (Figure 6.4). The integrations of the butyl terminus were compared with methyl protons associated with the bromo-endgroup, showing a 1:1 stoichiometric ratio of endgroups. GPC analysis of the bromo-terminated PDMS confirms a well-defined polymer with a $M_n$ of 3,300 g mol$^{-1}$ and a molecular weight distribution of 1.12. Good molecular weight agreement and narrow molecular weight distribution of the bromo-terminated PDMS indicate that the acid bromide reaction conditions were mild enough to preserve the integrity of the acid-sensitive PDMS backbone.
ATRP was used to synthesize PDMS-\(b\)-PtBA copolymers from the bromo-terminated PDMS (Figure 6.5). In this polymerization, free radicals are generated from a reversible redox process involving the bromo-endgroup of the PDMS macroinitiator and copper(I) bromide catalyst. The PMDETA ligand was used to solubilize the copper(I) bromide in the reaction mixture. The macroinitiator is activated when the copper(I) bromide abstracts the bromine atom from the PDMS endgroup, forming a stable tertiary radical that reacts with the tert-butyl acrylate monomer. During polymerization, the copper(II) bromide reversibly deactivates the propagating chain end of the copolymer, which reduces the concentration of radicals in the system and minimizes premature termination. Literature reports that narrow molecular weight distribution polymers can be synthesized using ATRP. In the synthesis of PDMS-\(b\)-PtBA, the reaction mixtures were thoroughly deoxygenated to maintain the activity of the copper(I)
bromide catalyst in solution.

[A diagram of the reaction between a bromo-terminated PDMS macroinitiator and tert-butyl acrylate, catalyzed by Cu(I)Br and PMDETA in toluene at 80°C.]

**Figure 6.5.** ATRP of tert-butyl acrylate using a PDMS macroinitiator.

ATRP of tert-butyl acrylate using a bromo-terminated PDMS macroinitiator successfully yielded well-defined block copolymers. Controlled acrylate block lengths of the PDMS-PtBA copolymers were achieved by changing the ratio of tert-butyl acrylate monomer to PDMS macroinitiator. Polymerizations were allowed to proceed for 12 h, after which high conversions of monomer (~7,000 g mol⁻¹) were observed. The polymerization mixture was diluted with chloroform and passed through an alumina column to remove the copper catalyst. Unreacted monomer was removed from the sample by heating under vacuum, resulting in an optically clear product. ¹H NMR analysis of the reaction product confirmed the polymerization of tert-butyl acrylate from a PDMS macroinitiator. A representative spectrum of a 3kPDMS-b-7.2kPtBA is shown in **Figure 6.6**.
Figure 6.6. \(^1\)H NMR spectrum of a 3kPDMS-\(b\)-7.2kPtBA.

GPC analysis was performed on the PDMS-\(b\)-PtBA samples. A representative GPC trace overlay comparing the 3,300 g mol\(^{-1}\) bromo-terminated PDMS macroinitiator to a 3kPDMS-\(b\)-7.2kPtBA is shown in Figure 6.7. This figure illustrates that the block copolymers synthesized via ATRP are monomodal with narrow molecular weight distributions, which is indicative of a living free radical polymerization.
Figure 6.7. GPC refractometer traces of 3kPDMS macroinitiator (gray) and 3kPDMS-\textit{b}-7.2kPtBA (black). ATRP yields block copolymers with monomodal peaks and narrow distributions.

\textbf{Table 6.1} contains the molecular weight and block length analysis by $^1$H NMR and GPC of the PDMS-\textit{b}-PtBA samples. Good agreement was found between both methods of analysis. This data suggests that ATRP is a suitable technique for synthesizing well-defined PDMS-\textit{b}-PtBA copolymers with good molecular weight control.
Table 6.1. PDMS-\textit{b}-PtBA molecular weights.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>PDI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^1$H NMR PDMS</td>
<td>$^1$H NMR PtBA</td>
</tr>
<tr>
<td>Monovinyl-PDMS</td>
<td>3,000</td>
<td>N/A</td>
</tr>
<tr>
<td>Bromo-PDMS</td>
<td>3,150</td>
<td>N/A</td>
</tr>
<tr>
<td>3kPDMS-\textit{b}-2.3kPtBA</td>
<td>3,000</td>
<td>2,300</td>
</tr>
<tr>
<td>3kPDMS-\textit{b}-3.6kPtBA</td>
<td>3,000</td>
<td>3,600</td>
</tr>
<tr>
<td>3kPDMS-\textit{b}-7.2kPtBA</td>
<td>3,000</td>
<td>7,200</td>
</tr>
</tbody>
</table>

*PDI = Polydisperity Index

Synthesis of PDMS-\textit{b}-poly(acrylic acid) from PDMS-\textit{b}-PtBA required a deprotection reaction that maintained the stability of the acid- and base-sensitive PDMS backbone in the block copolymers. Using a procedure adapted from Wu et al., the \textit{tert}-butyl ester groups of the PDMS-\textit{b}-PtBA copolymers were cleaved with ZnBr$_2$ as the deprotecting reagent (Figure 6.8).$^{174}$ The conditions of this deprotection reaction have been reported to be a mild alternative to other commonly used deprotecting reagents for \textit{tert}-butyl esters (i.e. trifluoroacetic acid).

PDMS-\textit{b}-PtBA copolymer was dissolved in dichloromethane and were stirred overnight in the presence of a large excess of ZnBr$_2$, forming complexes between the \textit{tert}-butyl ester groups of the copolymer and the deprotecting reagent. Water was added to the slurry, resulting in hydrolysis of the complexed \textit{tert}-butyl ester groups. PDMS-\textit{b}-poly(acrylic acid) copolymer was recovered by washing the reaction mixture three times with dichloromethane and concentrating the product under vacuum.
Figure 6.8. Cleavage of tert-butyl ester groups on PDMS-\textit{b}-PtBA using ZnBr$_2$ as the deprotecting reagent.$^{174}$

$^1$H NMR analysis was performed on the deprotected PDMS-\textit{b}-poly(acrylic acid) product (Figure 6.9). Disappearance of the tert-butyl ester groups (~1.3 ppm) was observed in the spectrum of the deprotected material. Figure 6.10 shows a GPC trace overlay of a PDMS-\textit{b}-PtBA and the corresponding PDMS-\textit{b}-poly(acrylic acid). GPC molecular weight analysis revealed an appropriate decrease in molecular weight between the PDMS-\textit{b}-PtBA (11,300 g mol$^{-1}$) and the PDMS-\textit{b}-poly(acrylic acid) (6,500 g mol$^{-1}$). The GPC trace of the deprotected material was monomodal with a narrow distribution similar to the PDMS-\textit{b}-PtBA precursor, indicating that no PDMS chain cleavage occurred during the ZnBr$_2$ deprotection reaction.
Figure 6.9. $^1$H NMR overlay of 3kPDMS-$b$-7.2kPtBA (black) and the corresponding deprotected 3kPDMS-$b$-3.5kPoly(acrylic acid) (gray).

Figure 6.10. GPC refractometer traces of 3kPDMS-$b$-7.2kPtBA (black: $M_n = 11,300$ g mol$^{-1}$; PDI = 1.25) and corresponding deprotected 3kPDMS-$b$-3.5kPoly(acrylic acid) (gray: $M_n = 6,500$ g mol$^{-1}$; PDI = 1.31) show that the deprotection reaction does not cause PDMS chain cleavage.
Figure 6.11 shows the DSC characterization of a 3kPDMS-b-7.2kPtBA. Two glass transition temperatures are observed (~ -128 °C and ~29 °C) during the 2nd heating cycle at 5 °C/minute, indicating a phase-separated material. Also, a small melting point transition is observed ~ -50 °C indicating a small amount of crystallinity in the PDMS phase.

Figure 6.11. DSC analysis of 3kPDMS-7.2kPtBA shows a phase-separated material. The trace shows the 2nd heating cycle at 5 °C/minute.

6.4 Conclusions
Dimethylvinylsilyl-terminated PDMS was synthesized via the living anionic polymerization of D₃. The vinylsilyl endgroup of this well-defined siloxane was functionalized with mercaptoethylamine hydrochloride, yielding a monoamino-terminated PDMS. ¹H NMR characterization of the monoamino-terminated PDMS showed quantitative addition of amine
functionality to the siloxane. The monoamino-PDMS could be used as a precursor for a wide range of reactions, producing novel well-defined PDMS systems. In this chapter, the monoamine-terminated PDMS was reacted with 2-isobromobutyryl bromide, yielding a monobromo-terminated PDMS macroinitiator for ATRP. A series of PDMS-\(b\)-PtBA copolymers were synthesized, and the characterization data showed good molecular weight agreement between \(^1\)H NMR and GPC methods of analysis with narrow molecular weight distributions indicative of living free radical polymerization. DSC analysis confirmed that the block copolymers were phase separated. A mild deprotection reaction was employed to cleave the tert-butyl ester groups, yielding PDMS-\(b\)-poly(acrylic acid) copolymers.

6.5 Acknowledgements

The authors are grateful for the financial support of the NSF/ARC Materials World Network for the Study of Macromolecular Ferrofluids (DMR-0602932 and LX0668968).
CHAPTER 7: Synthesis and Characterization of Polyionenes Based on Polydimethylsiloxane Soft Segments

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7.1 Introduction

Macromolecules containing quaternary amines in their backbone are commonly referred to as ionenes.26,185 These ammonium-containing polycations are synthesized using Menschutkin reaction-based step-growth polymerizations.186 In these polymerizations, quaternary ammonium salts are formed via the reaction between tertiary amines and alkyl halides.185,187 Previous work has focused on aliphatic polyionene systems, with interest focused on their antimicrobial properties.186-189

This chapter describes the synthesis and characterization of novel polyionenes containing polydimethylsiloxane soft segments. The goal of these PDMS-based ionenes was to design a film with well-defined ionic channels embedded in a hydrophobic PDMS matrix. Free-standing PDMS-based ionene films with varying soft segment contents and different hard segment groups were formed. The relationships between polyionene composition and the mechanical, thermal and morphological properties of the resulting films were studied.
7.2 Experimental

7.2.1 Materials
Celite (standard supercel, Alfa Aesar), 6-bromohexanoyl chloride (97%), 1,4-diazabicyclo[2.2.2]octane (DABCO, 98%), 1,4-dibromobutane (99%), sodium hydroxide (1 N, aq) and triethylamine (TEA, ≥ 99.5%) were purchased from Aldrich and used as received. Chloroform, diethyl ether and dichloromethane (EMD Chemicals, Drisolv) were used as received. Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. 1,4-Bis(bromomethyl)benzene (≥ 98%) and dimethylamine solution (60% by weight in water) were purchased from Fluka and used as received. α,ω-Aminopropyl-polydimethylsiloxanes (Gelest) were vacuum-stripped to remove cyclics prior to use. Magnesium sulfate (Mallinckrodt Chemicals, anhydrous powder) was used as received.

7.2.2 Synthesis
7.2.2.1 Bromoalkyl-terminated polydimethylsiloxane (PDMS)
A representative procedure for a 1982 g mol\(^{-1}\) bromoalkyl-terminated PDMS oligomer is provided. α,ω-Aminopropyl-terminated PDMS (20 g, 0.0118 mol) was dissolved in dry dichloromethane (300 mL) in a 500-mL, flame-dried, two-necked round-bottom flask fitted with an addition funnel. The reaction mixture was cooled to 0°C with an ice-water bath under a head of N\(_2\). TEA (3.84 g, 0.0379 mol) was added via syringe. A solution of 6-bromohexanoyl chloride (5.79 g, 0.027 mol) in dichloromethane (20 mL) was added to the addition funnel under N\(_2\). The acid chloride solution was added dropwise to the stirring PDMS solution. The mixture was allowed to warm to room temperature and reacted for 24 h with stirring. The reaction mixture was passed through a Celite column, followed by three washes with DI water. The
dichloromethane was removed under vacuum, and the difunctional bromoalkyl-terminated PDMS was dried at 60 °C under vacuum for 12 h. ¹H NMR was used to confirm the final product.

7.2.2.2 Dimethylamino-functional xylene

1,6-(N,N’-dimethylaminomethyl)benzene was prepared from a procedure adapted from Spencer et al.¹⁹⁰ 1,6-(Bromomethyl)benzene (6.0 g, 0.023 mol) was dissolved in THF (150 mL) in a round-bottom flask. The reaction mixture was cooled to -78 °C with an acetone/dry ice bath. A 60% aqueous solution of dimethylamine (397 mL, 4.37 mol) was added to the cooled reaction via syringe. The reaction mixture was stirred at -78 °C for 30 min, followed by stirring at room temperature for 4 days. The solvents were removed under vacuum, and the product was dissolved in diethyl ether (200 mL). The diethyl ether solution was washed with a 2 M solution of sodium hydroxide (3X), then DI water (3X). The diethyl ether layer was dried over magnesium sulfate, filtered and concentrated under vacuum, yielding 1,6-(N,N’-dimethylaminomethyl)benzene. ¹H NMR was used to confirm the final product (Figure A.1).

![Figure 7.1](image) ¹H NMR of 1,6-(N,N’-dimethylaminoethyl)benzene.
7.2.2.3 Polyionene synthesis

A characteristic procedure for synthesizing a PDMS ionene with a xylene hard segment is provided. A difunctional bromoalkyl-terminated PDMS oligomer (1982 g mol$^{-1}$, 5.1 g, 2.6 mmol) and 1,6-(N,N’-dimethylaminomethyl)benzene (0.50 g, 2.6 mmol) were dissolved in dry chloroform (18.5 mL) in a flame-dried round-bottom flask fitted with a reflux condenser. The reaction mixture (20 wt % solids) was refluxed at 75 °C for 24 h. Films were cast into Teflon molds and were air-dried for 24 h. The ionene films were then dried in a vacuum oven at 60 °C for 12 h.

7.2.3 Characterization

7.2.3.1 $^1$H NMR Spectroscopy

$^1$H NMR analysis of compounds was performed on a Varian 400 Unity NMR and a Varian Inova 400 NMR. Chloroform-d (99.8 atom %) was used as the solvent for all of the samples.

7.2.3.2 Gel Permeation Chromatography (GPC)

GPC analyses were performed on an Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set. The GPC data collected in chloroform at 30$^0$C was analyzed using a Universal calibration, allowing for determination of absolute molecular weights.

7.2.3.3 Dynamic mechanical analysis (DMA)

Tests were performed on a Seiko DMS 210 tensile module with an auto-cooler for precise temperature control. Rectangular samples were cut (12 mm in length and 4-6 mm in width) from cast films and deformed (1 Hz frequency, 10 mm amplitude) under a dry nitrogen atmosphere. The temperature was increased from -150 to 250 °C, at a heating rate of 2°C min$^{-1}$. 
7.2.3.4  Thermomechanical analysis (TMA)
Tests were performed on a TA Instruments TMA 2940. Square samples (ca. 4 mm\(^2\)) were cut from solvent cast films and loaded into the instrument with a preload force of 0.05 N. The temperature was ramped from room temperature at a heating rate of 2°C min\(^{-1}\) under a dry air atmosphere until the sample flowed.

7.2.3.5  Moisture uptake
Rectangular samples were cut from solvent cast films and dried at 40 °C under vacuum. The samples were then exposed to the ambient atmosphere for a week and their weight gain was measured with a Mettler Toledo AG204 digital balance.

7.2.3.6  Thermogravimetric analysis (TGA)
Tests were performed on a TA Instruments Q400 TGA. Samples were initially dried in the TGA at 120 °C for 15 min, then they were heated at 5°C min\(^{-1}\) from 30 to 700 °C in an air atmosphere.

7.2.3.7  Atomic force microscopy (AFM)
AFM images were obtained using a Veeco Dimension 3000 atomic force microscope with a Nanoscope IIIa controller. Images were obtained under a nitrogen atmosphere using Nanodevices TAP150 silicon cantilever probe tips (5 N/m spring constant, ~100 kHz resonant frequency). The free air amplitude was normally set at 4.0 V and the set point ratio was in the range of 0.4 to 0.7, which constitutes hard to medium tapping respectively. The phase images of the samples were obtained by performing AFM analysis on the free air side of the solvent cast films.

7.2.3.8  Tensile testing
Dog-bone samples were cut from solvent cast films and their tensile properties were measured under ambient conditions (50% RH and 26 °C) with an Instron 5500R machine controlled by Bluehill V2.1 software. Samples were elongated at 50 mm min\(^{-1}\) until failure.
7.3 Results and discussion

7.3.1 Synthesis of difunctional bromoalkyl-terminated PDMS oligomers

α,ω-Aminopropyl-functional PDMS oligomers that had been prepared in equilibration reactions were utilized as the precursors for the soft segments in the polyionenes discussed herein. The molecular weight distributions of such oligomers are well-known to be close to two due to the redistributions in molecular weights that occur throughout the syntheses.\textsuperscript{167,191-193} A distribution of chains and cyclic species result from these preparations, and the cyclics were removed by vacuum stripping at elevated temperature prior to any syntheses described in this work.

The aminopropyl endgroups were derivatized with 6-bromohexanoyl chloride to afford bromoalkyl endgroups that were suitable for the polyionene syntheses (Figure 7.2). It is noted that the linking group in the derivatization is an amide bond and that the amides are significantly more chemically stable against nucleophiles than an ester would be. Thus, these materials were well-designed to withstand the nucleophilic ionene formation reaction. A slight excess of triethylamine relative to acid chloride was utilized as an acid scavenger in these derivatizations to avoid any reaction of an acid by-product with the acid-sensitive PDMS backbone. Oligomers with 1982 and 3082 g mol\(^{-1}\) number average molecular weights were prepared to investigate polyionene properties as functions of the oligomer segment lengths. Molecular weights and distributions of the bromoalkyl-functional PDMS products were analyzed by NMR and GPC with good agreement between both methods (Table 7.1).
Figure 7.2. Synthesis of bromoalkyl-terminated PDMS.

Table 7.1. $\alpha,\omega$-Bromoalkyl-terminated PDMS molecular weights.

<table>
<thead>
<tr>
<th>Polymer*</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>PDI**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS (1700)</td>
<td>1982</td>
<td>2.1</td>
</tr>
<tr>
<td>PDMS (2800)</td>
<td>3082</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* The numbers in this column refer to the number average molecular weights of the $\alpha,\omega$-aminopropyl-terminated PDMS precursors as determined by $^1$H NMR

** PDI = Polydispersity Index

A representative $^1$H NMR spectrum demonstrates the quantitative addition of 6-bromohexanoyl chloride to a 1700 g mol$^{-1}$ aminopropyl-terminated PDMS (Figure 7.3). The polydispersity indices were close to two as expected.
7.3.2 Polyionene synthesis

PDMS-based polyionenes were prepared in step-growth polymerizations by chain extending the bromoalkyl-functional oligomers and either 1,6-(N,N'-dimethylaminoethyl)benzene or diaminobicyclooctane (DABCO) in Menshutkin reactions (Figure 7.4). Careful attention was given to maintaining 1:1 stoichiometries between the dimethylamine and bromoalkyl groups to obtain high molecular weights. Dibromobutane was also employed in select cases to increase the hard segment length. Free-standing, optically clear films containing different compositions of PDMS soft segments were prepared from solutions of the ionene polymers (Table 7.2).
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Figure 7.4. Synthesis of PDMS-ionenes containing DABCO hard segments.

Table 7.2. Series of PDMS-based ionenes synthesized in step-growth polymerizations. Compositional data was obtained from NMR spectra and char yields were determined by TGA. (CE = PDMS-based ionene chain extended with dibromobutane to increase hard segment length)

<table>
<thead>
<tr>
<th>Polymer*</th>
<th>Weight % PDMS</th>
<th>% Char Yield</th>
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</thead>
<tbody>
<tr>
<td>Xylene-PDMS(1700)</td>
<td>78</td>
<td>14</td>
</tr>
<tr>
<td>DABCO-PDMS(1700)</td>
<td>80</td>
<td>33</td>
</tr>
<tr>
<td>DABCO-PDMS(2800)</td>
<td>87</td>
<td>32</td>
</tr>
<tr>
<td>DABCO-CE-PDMS(1700)</td>
<td>70</td>
<td>19</td>
</tr>
</tbody>
</table>

*The numbers in this column correspond to the number average molecular weights of the a,ω-aminopropyl-terminated PDMS precursors

7.3.3 Temperature-dependent moduli of PDMS-based ionenes

Dynamic mechanical spectra of solvent-cast PDMS-based ionene films reflect the effect of ionene composition on the temperature-dependent modulus profiles (Figure 7.5). A tan δ peak at ca.-120°C due to the glass transition of the PDMS soft segment was observed for all of these
materials. Trends in the DMA profiles reflect the hard segment content of the ionenes. The PDMS-based ionenes with the lower molecular weight (~1700 g mol\(^{-1}\)) oligomer have similar concentrations of hard segments (~22 wt% for the material prepared from the dibromoxylene and ~ 20 wt% for DABCO), but the xylene-containing ionene exhibited a lower rubbery plateau modulus and dissociates at a lower temperature relative to the DABCO-containing ionene. This could be attributed to increased packing efficiency due to the rigidity of the DABCO segments.

![Figure 7.5](image.png)

**Figure 7.5.** Dynamic mechanical spectra for films of various PDMS-based polyionenes.
The hard segment content of the DABCO-containing ionenes was varied by increasing the molecular weight of the PDMS from ~1700 to ~2800 g mol\(^{-1}\), thus reducing the % hard segment from ~20 to ~13 wt%, respectively. Incorporation of dibromobutane as a chain extender into the ~1700 g mol\(^{-1}\) PDMS-based ionene increased the hard segment to ~30 wt%. DMA showed that the intensity of the PDMS \(T_g\) was inversely proportional to the amount of hard segment. Both rubbery plateau region and dissociation temperature of the hard domains increase with ionene HS content.

### 7.3.4 Thermo-mechanical analysis of PDMS-based ionenes

The softening temperatures of the various ionenes were verified through TMA under a nitrogen atmosphere (Figure 7.6). The softening points were found to be similar to those obtained from DMA. The upturn in the DABCO-CE-PDMS spectrum was likely due to thermal degradation and subsequent evolution of the monomer. The degradation temperature of these ionene films was ~200 °C.
7.3.5 Morphologies of PDMS-based ionenes

The high ambient rubbery moduli of the PDMS polyionenes indicated the presence of a phase-separated morphology, which was investigated further by ambient tapping mode AFM under nitrogen (Figure 7.7). A phase-separated morphology, with thread-like hard segment domains dispersed in the PDMS soft matrix was observed for the ionene films. The DABCO-PDMS(1700) ionene (Figure 7.7b) was found to have better phase separation than the xylene-PDMS(1700) ionene (Figure 7.7a). As the PDMS molecular weight was increased from 1700 to 2800 g mol\(^{-1}\) (Figure 7.7d) in the DABCO-containing ionenes the AFM image revealed a softer film with fewer thread-like HS domains. AFM showed that incorporation of dibromobutane chain-extender in the DABCO-containing ionene (Figure 7.7c) led to an increase in HS content with smaller thread-like HS domains compared to the images of the ionenes.
Figure 7.7. Tapping mode AFM phase images of PDMS polyionenes at room temperature: a. xylene-PDMS (1700); b. DABCO-PDMS (1700); c. DABCO-CE-PDMS (1700); d. DABCO-PDMS (2800).

7.3.6 Tensile properties of PDMS-based ionenes

Uniaxial tensile analysis of the PDMS-based ionenes was conducted under ambient conditions (Figure 7.8). Increasing the HS content in the DABCO-containing ionenes lead to a higher modulus. The xylene-containing ionene displayed a higher modulus than the corresponding DABCO-containing ionene (1700 g mol⁻¹ PDMS). The high yield point observed
for the DABCO-CE-PDMS(1700) ionene indicates that there is long-range connectivity between the hard domains.

![Figure 7.8. Tensile properties of PDMS-based ionenes.](image)

### 7.4 Conclusions

PDMS-based ionenes were synthesized by reacting dibromoalkyl-terminated PDMS with 1,6-(N,N’-dimethylaminomethyl)benzene or 1,4-diazabicyclo[2.2.2]octane). Free-standing transparent films with different compositions were synthesized and the mechanical, thermal and morphological properties were investigated.

### 7.5 Acknowledgements

This material is based upon work supported in part by the U.S. Army Research Office under grant number W911NF-07-1-0452 Ionic Liquids in Electro-Active Devices (ILEAD) MURI. The authors are also grateful for the financial support of the NSF Materials World Network under contract number DMR-0602932

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CHAPTER 8: Conclusions and Recommendations for Future Work

Polyether dispersion stabilizers containing different functional anchor groups were synthesized. These stabilizers were used to coat monodisperse magnetite nanoparticles in a controlled fashion. In one study, two molecular weights of dimethylvinylsilyl-PEO-OH were modified to contain a monocarboxylic acid, monoammonium or monozwitterionic phosphonate anchor group. PEO-magnetite complexes with similar polymer loadings were synthesized from this series of stabilizers. The colloidal stability of these complexes was studied in aqueous media and physiological media using DLS. The monocarboxylic acid and monoammonium stabilizers exhibited time-dependent colloidal stability in phosphate buffered saline (PBS). However the monozwitterionic phosphonate stabilizer showed improved colloidal stability in PBS. In another study, a series of triammonium PPO-\(b\)-PEO-OH stabilizer were synthesized and the colloidal stabilities of the corresponding magnetite complexes were studies. Based on the results, it was proposed that the addition of a PPO block may help improve the colloidal stability of the polyether-magnetite complexes in PBS. Additionally, the triammonium anchor group improved the colloidal stability of magnetite complexes over the monoammonium anchor group.

Recommendations for future work pertaining to polyether-magnetite complexes are twofold. First, functionalize trivinylsilyl-PEO-OH to contain a trizwitterionic phosphonate anchor group. Complexes containing the monozwitterionic phosphonate anchor group exhibited the best colloidal stability in physiological media. Three zwitterionic phosphonate anchor groups may further improve this colloidal stability. Second, synthesize trizwitterionic phosphonate-PPO-PEO-OH stabilizers and determine if these show improved stability over the trizwitterionic phosphonate-PEO-OH stabilizers. Completion of these two studies should advance our
understanding of how anchor group and polyether composition relate to the colloidal stability of polyether-magnetite complexes.

One-part PDMS-magnetite nanoparticle fluids were synthesized using a high shear process and magnetic separation techniques. These one-part fluids are unique in the fact that they do not require the addition of a non-functional PDMS oligomer solvent to generate a magnetic hydrophobic fluid. A series of PDMS-magnetite nanoparticle fluids containing different molecular weight stabilizers were synthesized. A magnetic separation study was performed to determine if PDMS molecular weight influences the magnetic separation profiles of the fluids. It was found that larger particles containing lower polymer contents were preferentially separated from the bulk material, independent of PDMS stabilizer molecular weight. Experimental PDMS-magnetite complex sizes determined by DLS more closely match theoretical complexes sizes obtained from a density distribution model with increasing number of magnetic separations. It was found that the slight aggregation that occurs in the one-part PDMS-magnetite nanoparticle fluids makes it difficult to analyze SANS results.

Future work on the PDMS-magnetite complexes should focus on decreasing the aggregation of the particles in the fluid. This could be achieved by using the monodisperse magnetite nanoparticles that are described in Chapters 3 and 4. This should alleviate the need for magnetic separations and make characterization of the fluids easier. Additionally, incorporating some of the novel PDMS modifications described in Chapter 6 (i.e. addition of amine functionality to a vinylsilyl-terminated PDMS) into the PDMS magnetite dispersion stabilizers could improve the integrity of the hydrophobic magnetite fluid.

Well-defined PDMS-\textit{b}-PtBA and PDMS-\textit{b}-poly(acrylic acid) copolymers were synthesized using living free radical techniques from novel PDMS precursors (i.e.
monofunctional amine-terminated PDMS and monobromo-terminated PDMS). This series of copolymers were extensively characterized at each synthetic step, illustrating the relative ease and versatility of this procedure. Future work should involve synthesizing copolymers with different molecular weight PDMS macroinitiators, as well as attempting to modify the poly(acrylic acid) block with 4-vinyl benzyl chloride to produce a crosslinkable siloxane hydrogel.

PDMS-based ionenes with different hard segment groups were synthesized and extensively characterized. Recommendations for future work include continuing to study the relationship between ionene composition and mechanical, thermal and morphological properties.
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