ABSTRACT

BENNER, STEVEN. Development of a Coarse-Grained Model of Chitosan for Predicting Solution Behavior. (Under the direction of Dr. Carol K. Hall).

We have developed a new, implicit-solvent, coarse-grained model of chitosan designed for use in discontinuous molecular dynamics (DMD) simulations. The parameters for this model were developed using a multi-scale modeling approach based on explicit-solvent, atomistic simulations. We used our coarse-grained model of chitosan to predict self-assembly behavior as a function of the degree of acetylation (DA) and amount of hydrophobic modification (based on alkanes). The self-assembly of chitosan was studied to determine its potential as a biocompatible material for targeted drug delivery and oil spill remediation.

Our first study focused on using HMC as an oil dispersant additive for applications in oil spill remediation. We used a simple coarse-grained model of oil and HMC (not based on atomistic simulations) to investigate how modification chain length and modification density inhibit oil aggregation over time. We investigated 4%, 12%, and 20% modified chitosan with modification chain lengths of 5 or 15 spheres. Results show that all HMCs regardless of architecture helped reduce the overall extent of oil aggregation over time. For HMCs with 5-sphere modification chains, increasing the modification density resulted in an increase in the oil’s solvent accessible surface area (SASA) for each modification density tested. For HMCs with 15-sphere modification chains, increasing the modification density from 4% to 12% resulted in an increase in the oil SASA, however an increase in modification density from 12% to 20% did not show any noticeable increase in oil SASA. This demonstrated that there
is a saturation density of longer modification chains, above which there is no improvement in performance as an oil dispersant additive. We also determined that longer modification chains more effectively penetrate into oil droplets, resulting in a deformation of their shape which was quantified by measuring the asphericity of the oil droplets.

Next we performed a multi-scale modeling procedure to develop a more detailed coarse-grained model of chitosan. The model contains two different monomer types which allow the investigation of systems with varying DA: protonated glucosamine (GlcN\(^{+}\)) and N-acetylglucosamine (GlcNAc). Each monomer is modeled with three coarse-grained sites; the chitosan chain length was set to 100 monomers. The results showed increased association between chitosan chains with increasing DA. Increasing the DA from 10% to 50% resulted in the formation of percolated chitosan networks earlier in time for each increase in DA. Furthermore, the networks formed at DA’s of 20% and higher contained all of the chains in the system. The number of monomer-monomer interactions also increased with increasing DA, indicating a stronger network with more structural integrity. Under dilute solution conditions, our model closely matches the radius of gyration and persistence lengths of chitosan reported in experiments as a function of DA. Overall, we have shown that the behavior of our coarse-grained model conceptually matches several behaviors observed experimentally.

To understand the effect of chitosan composition on self-assembly in solution, we simulated chitosan with three different sequences of acetylated monomers: random, evenly spaced, and blocky (5-monomer blocks) at DA’s ranging from 10% to 50%. Our results
show that the sequence of acetylated monomers can have a significant impact on the rate of network formation, particularly at low DA. At 10% DA, we showed that only a blocky sequence of acetylated monomers leads to a stable percolated structure while random and evenly spaced sequences do not. For DA’s of 20% and higher, the blocky sequences form a percolated network the fastest, followed by random, and then evenly spaced sequences. We also showed that the pore size distribution of the chitosan networks can be adjusted based on both the DA and the monomer sequence. Increasing DA leads to networks with larger pores due to the increased hydrophobic association between chains. Blocky sequences of acetylated monomers lead to networks with larger pores than random and evenly spaced sequences at a set DA. These different pore size distributions lead to changes in the diffusion of molecules inside the network. We show that networks of chitosan at high DA allow diffusion of larger molecules than networks at low DA due to the increase in pore size.

Finally we focused on the self-assembly of HMC in solution as a function of modification chain length, modification density, and the presence of hydrophobic nanoparticles in solution. For these simulations we extended our model to include representations for hydrophobic modification chains based on alkanes. We performed simulations of 5% and 10% modified HMC with modification chains lengths of 4, 6, and 8 spheres. The results indicate that increasing the modification chain length and modification density lead to increased network formation at an HMC concentration of 1.5 weight percent. We then performed simulations of the same HMC architectures at a concentration of 1.0 weight percent in the presence of hydrophobic nanoparticles with diameters of 40, 60, and 80
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Development of a Coarse-Grained Model of Chitosan for Predicting Solution Behavior

by

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DEDICATION

This dissertation is dedicated to my parents, Bill and Susan Benner, my brother, Glenn Benner, and my fiancé, Maggie Fuentes. Thank you for keeping me motivated throughout this process and keeping things in perspective for me when I needed it. Your support has helped me more than I can explain, and I could not imagine going through this process without you.
**BIOGRAPHY**

Steven Benner was born in 1989, in Allentown, PA. He is the son of Bill and Susan Benner, and the younger brother of Glenn Benner. In 2016 he became engaged to Maggie Fuentes. He attended the Pennsylvania State University in University Park, PA and earned a Bachelor of Science in Chemical Engineering in 2011. During his undergraduate studies he worked as an intern for three summers at the pharmaceutical company Sanofi Pasteur in Swiftwater, PA, and as an undergraduate research assistant in the Department of Chemical Engineering under Dr. Andrew Zydney. Upon completion of his Bachelor of Science degree, he began his graduate studies at North Carolina State University in the Department of Chemical and Biomolecular Engineering in the Fall of 2011, advised by Dr. Carol Hall. During the pursuit of his Ph.D. he also completed an internship with the pharmaceutical company Boehringer Ingelheim in Ridgefield, CT.
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CHAPTER 1

Motivation and Overview
1.1 Motivation

Self-assembled systems have shown promise in applications such as environmental remediation\textsuperscript{1-4}, materials science\textsuperscript{5-7}, and biotechnology\textsuperscript{8-10}. These materials are designed on the molecular level to have a desired functionality, and therefore a controlled assembly in solution. One of the main benefits of self-assembled systems is that structures can be formed on smaller length scales than can be done through traditional manufacturing techniques. These systems can even be designed to respond to stimuli such as changes in pH\textsuperscript{11,12}, temperature\textsuperscript{13,14}, and electric fields\textsuperscript{15,16}. Self-assembled systems based on biocompatible and biodegradable polymers have gained popularity due to their minimal environmental impact and their ability to be used inside the human body. Chitosan is of particular interest because it is both biocompatible and biodegradable, it consists of two monomer types, it is typically charged in solution, and it can be easily modified chemically. For these reasons, chitosan has been used in a wide variety of applications ranging from oil dispersant additives to drug carriers.

Chitosan is a versatile polysaccharide derived from chitin, which is the structural component in the exoskeleton of crustaceans such as crabs and shrimp. Chitosan consists of a random sequence of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) monomers, a result of the deacetylation of chitin (which consists of only GlcNAc monomers).\textsuperscript{17,18} The combination of these two monomers gives chitosan a unique behavior in solution. The primary amine group on the GlcN monomers is protonated (GlcN\textsuperscript{+}) at a pH less than or equal to its pKa (\textasciitilde 6.5), giving the monomer a positive charge, leading to
repulsion between monomers. The acetyl group on the GlcNAc monomers causes hydrophobic and hydrogen bonding interactions between monomers, resulting in net attractive interaction between monomers. Therefore, chitosan solution behavior is controlled by a balance of electrostatic repulsions between GlcN\(^+\) monomers and hydrophobic attractions between GlcNAc monomers. Chitosan can also be hydrophobically-modified via a reductive amination reaction of an alkyl aldehyde with the primary amine group of the GlcN monomers. A tool that could quickly investigate various combinations of these parameters would be valuable for designing self-assembled materials based on chitosan. One approach that is often used to gain insight into molecular level behavior is computer simulation.

Molecular dynamics (MD) simulations have been used for years to understand how molecular level behavior can affect large-scale properties. Molecular simulations can be divided into two main categories: all-atom and coarse-grained. All-atom simulations account for the motion of every atom in the system, including solvent atoms. This level of detail provides accurate results, but is computationally expensive. Calculating the interactions between tens or even hundreds of thousands of atoms is time consuming, and precludes the ability to study large systems and long time scales. Coarse-grained modeling addresses this problem by grouping several atoms into a single coarse-grained site; each site is meant to represent the behavior of the group of atoms that it contains. This procedure reduces the total number of sites whose dynamics must be calculated, and simplifies the geometry and energetics of the molecules in the system. These simplifications allow the simulation of
larger systems over longer time scales than can be achieved through traditional all-atom molecular simulations.

Traditional MD simulations are computationally expensive, even when using coarse-grained models. The interaction potential between two coarse-grained sites is a continuous function of the separation distance between the two sites, e.g. the Lennard-Jones potential. Therefore, the force on each site must be recalculated with every time step to capture the energetics of the system. A small, constant time step is required with a continuous potential because even slight changes in position can result in significant changes to the system energy. If the time step is too large, important dynamic information can be missed, leading to inaccurate results. Therefore, it would be desirable to have a method in which the system can advance through time more quickly, without losing the fundamental behavior of the molecules in the system.

Discontinuous molecular dynamics (DMD) simulations can be used in place of traditional MD to alleviate some of the time scale limitations associated with traditional MD. DMD simulations use discontinuous interactions potentials such as hard-sphere, square-well, or square-shoulder potentials to describe potential energy changes as a function of the distance between two atoms, in contrast to the continuous potentials implemented in traditional MD. The only change in potential energy between two atoms occurs when they reach a separation distance corresponding to a discontinuity in their potential energy function. Since the atoms in a DMD simulation move linearly, the time until two atoms reach a discontinuity in their potential energy function can be calculated. The algorithm searches for the pair of atoms that will reach a discontinuity in their potential energy function.
the soonest, advances to that time, and analytically updates the collision dynamics. Therefore, the simulation is advanced from collision to collision rather than by a small, constant time step.\textsuperscript{20} 

An efficient simulation technique implemented to understand the solution behavior of chitosan as a function of its composition could be used to design materials for a variety of applications. The work in this thesis focuses on development of a novel coarse-grained model of chitosan for use in DMD simulations that can be used to study self-assembly of chitosan in solution.

1.2 Overview

In this section we summarize the remainder of the dissertation. Each chapter contains a literature review and references.

1.2.1 Simulation Study of Hydrophobically-modified Chitosan as an Oil Dispersant Additive

Chapter 2 describes the use of hydrophobically-modified chitosan (HMC) as an oil dispersant additive, more specifically, how the architecture of HMC affects its ability to prevent oil aggregation. The backbone length of the HMC is held constant at 50-spheres, while the modification chain length is adjusted to 5 or 15 spheres, and the modification density (the percentage of chitosan spheres with attached hydrophobic modification chains) is adjusted to 4\%, 12\%, and 20\%. Results show that HMCs with 15-sphere modification
chains are more effective at maximizing the oil’s solvent accessible surface area (SASA) than HMCs with 5-sphere modification chains for each of the modification densities tested. Increasing the modification density of HMC with 5-sphere modification chains also increases the surface area of oil over time for each modification density tested. Increasing the modification density of HMCs with 15-sphere modification chains from 4% to 12% results in an increase in the oil’s SASA, however there is negligible change in the oil’s SASA when the modification density is increased from 12% to 20%. This shows that there is a saturation density of long modification chains, above which there is no further improvement in performance. We also show that HMCs with 15-sphere modification chains deform the shape of the oil droplets as a result of the modification chains penetrating deeply into the droplet, while HMCs with 5-sphere modification chains do not significantly change the shape of the oil droplets.

1.2.2 Development of a Coarse-Grained Model of Chitosan for Predicting Solution Behavior

Chapter 3 discusses a multi-scale modeling approach used to design a novel implicit-solvent coarse-grained model of chitosan for use in discontinuous molecular dynamics (DMD) simulations. The parameters for this model were derived from explicit-solvent atomistic molecular dynamics simulations of chitosan molecules in water. The model includes two monomer types, protonated glucosamine (GlcN\(^+\)) and N-acetylglucosamine (GlcNAc), where each monomer is represented by three coarse-grained sites. Interaction
potentials were derived using an iterative Boltzmann-inversion technique, and geometric constraints were satisfied based on bond length and angle distributions observed from atomistic simulations. The model was able to closely match chitosan gel formation data as a function of the degree of acetylation (DA). Increasing DA led to a decrease in the time needed to form a percolated network, indicating a decrease in the time needed to form a gel, as was observed experimentally. We also showed that all of the chains in the system assembled into a single network. An analysis of monomer-monomer interactions showed that the interactions between two GlcNAc monomers dominated chitosan self-assembly in solution. Simulations of chitosan under dilute solution conditions showed that our model closely reproduces the radius of gyration (Rg) and persistence lengths (Lp) from several experimental studies.

1.2.3 Effect of Monomer Sequence and Degree of Acetylation on the Self-assembly and Porosity of Chitosan Networks in Solution

Chapter 4 investigates how the sequence of N-acetylg glucosamine (GlcNAc) monomers along the chitosan chain affects its self-assembly in solution. We investigated three different monomer sequences: random, evenly-spaced, and blocky sequences of GlcNAc monomers, where the blocky sequences contained blocks of five GlcNAc monomers in a row spaced evenly along the chain. The results show that a blocky sequence of acetylated monomers leads to a percolated network earlier in time than both random and evenly spaced networks. The random sequence of acetylated monomers forms a percolated
network later than a blocky sequence, but earlier than an evenly spaced sequence because a random sequence contains both blocky and evenly-spaced regions. We also show that increased blockiness in the sequence of GlcNAc monomers leads to more GlcNAc-GlcNAc and GlcNAc-GlcN\(^+\) monomer associations than less blocky sequences. The pore size distributions of networks with different degrees of acetylation (DA) and monomer sequences were also calculated. The results indicate that increasing DA leads to chitosan networks with larger pores because the chains associate more strongly due to the increased hydrophobic interaction between GlcNAc monomers. Blocky sequences of acetylation lead to slightly larger pore size distributions than random and evenly spaced sequences at DA’s below 30%, while all sequences have nearly identical pore size distributions at DA’s above 30%. Finally, we calculated the mean squared displacement of hard-sphere molecules with diameters of 20 Å and 60 Å inside networks consisting of 10% DA and 50% DA chitosan with blocky sequences of GlcNAc monomers. We show that the large pores in a 50% DA network allow equal rates of diffusion of both particles. However, we see that a 10% DA network results in faster diffusion of the 20 Å particle than the 60 Å particle.

1.2.4 Nanoparticle Induced Assembly of Hydrophobically-Modified Chitosan

Chapter 5 discusses how the addition of hydrophobic modifications to chitosan affects its self-assembly in solutions with and without the presence of hydrophobic nanoparticles. First we investigate systems of 1.5 wt% chitosan that are 5% and 10% modified with 4-, 6-, and 8-sphere hydrophobic modification chains in the absence of
nanoparticles. The results show that 5% modified HMCs only form a stable percolated network in solution with 8-sphere modification chains but not with 4- and 6-sphere modification chains. When the modification density is increased to 10%, stable percolated networks form for HMCs with 6- and 8-sphere modification chains, but not with 4-sphere modification chains. We also see that the pore size distributions of the HMC networks are the same for all modification chain lengths and modification densities, except for 10% modified HMCs with 8-sphere modification chains, which have larger pore sizes due to increased chain hydrophobicity. Next we added 4, 6, and 8 nm diameter hydrophobic nanoparticles at a concentration of 2.0 wt% to HMC solutions of varying modification densities and modification chain lengths at an HMC concentration of 1.0 wt%. Results indicate that the addition of hydrophobic nanoparticles leads to the formation of stable percolated networks while HMCs alone do not. This occurs because the nanoparticles act as junction points that can interact with several HMC molecules at a time. We also see that having a greater number of small nanoparticles leads to the formation of networks earlier in time than having fewer large nanoparticles. The nanoparticles act as association sites that attract several modification chains from different chitosan molecules to a common location.

1.2.5 Future Work

In chapter 6 we provide insight into some interesting future studies regarding the self-assembly of chitosan in solution. We discuss the application of ionic cross-linking of chitosan to control the formation of nanoparticles for drug delivery. We also discuss
parameterizing pH-induced swelling of chitosan hydrogels and parameterizing drug molecules to fit into the framework of our model to study controlled drug delivery using chitosan nanoparticles.

1.3 Publications

Chapters 2-5 are based on the following publications:


Chapter 5: S. W. Benner and C. K. Hall, “Nanoparticle Induced Assembly of Hydrophobically-Modified Chitosan”, submitted
1.4 References


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CHAPTER 2

Simulation Study of Hydrophobically-modified Chitosan as an Oil Dispersant Additive

Chapter 2 is essentially a manuscript by Steven W Benner, Vijay T John, and Carol K Hall accepted by the Journal of Physical Chemistry B.
Simulation Study of Hydrophobically-modified Chitosan as an Oil Dispersant Additive

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Abstract

Hydrophobically-modified chitosan (HMC) is being considered as a possible oil dispersant additive to reduce the volume of dispersant required in oil spill remediation. We present the results of discontinuous molecular dynamics (DMD) simulations intended to determine how the HMC architecture affects its ability to prevent oil aggregation. The HMCs have a comb copolymer architecture with hydrophobic side chains (modification chains) of various lengths (5 – 15 spheres) to represent alkane chains that are attached to the chitosan backbone. We calculated the oil’s solvent accessible surface area (SASA), aggregate size distribution, and aggregate asymmetry at various values of the HMC modification chain length, modification density, and concentration to determine HMC efficacy. HMCs with long modification chains result in larger oil SASA than HMCs with short modification chains. For long modification chains there is no increase in oil SASA with increasing modification density above a saturation value. The size distribution of the oil aggregates
depends on the modification chain length; systems with long modification chains lead to large aspherical aggregates while systems with short modification chains lead to small tightly packed aggregates. A parametric analysis reveals that the most important factor in determining the ability of HMCs to prevent oil aggregation is the interaction between the HMC’s modification chains and the oil molecules, even when using short modification chains. We conclude that HMCs with long modification chains are likely to be more effective at preventing oil aggregation than HMCs with short modification chains, and that long modification chains impede spherical oil droplet formation.

2.1 Introduction

Oil spills have caused major environmental disasters over the past 50 years, the two most notable being the Exxon Valdez spill in 1989 and the recent Deepwater Horizon spill in 2010. Dispersants were used following the Deepwater Horizon spill in an effort to prevent oil slicks, and to promote the natural degradation of oil by ocean bacteria, but there was significant debate over their toxicity and effectiveness. Dispersants such as Corexit 9500A work by lowering the interfacial tension between oil and water and hence promote mixing, a process that is assisted by mechanical input from wind or wave energy. This results in the formation of much smaller oil droplets than would otherwise have occurred. If there was a way to stabilize the small oil droplets before they have the chance to re-coalesce, this would enhance the dispersants’ effectiveness because it would increase the surface area of oil exposed to hydrocarbon-degrading bacteria. In this paper we
consider a viable option for a biocompatible oil dispersant additive, hydrophobically-modified chitosan (HMC), which has been suggested to be both environmentally friendly and effective at preventing oil coalescence.

We are investigating the efficacy of HMCs as an oil dispersant additive to stabilize small oil droplets in water for use in oil spill remediation to potentially reduce the amount of dispersant required. Chitosan is a linear polysaccharide composed of a mixture of D-glucosamine and N-acetyl-D-glucosamine monomers derived from chitin, a naturally-occurring polymer found in the exoskeletons of ocean crustaceans such as crabs and shrimp. Chitosan can be hydrophobically modified through a reductive amination reaction, giving it an amphiphilic functionality. Previous studies have explored the behavior of chitosan and hydrophobically-modified chitosan in applications such as drug delivery, tissue engineering, gelation and network formation, and flocculating agents for oil/water emulsions. The idea of using HMCs as oil stabilizers was introduced by Venkataraman and coworkers. In their experiments, chitosan was hydrophobically modified with alkane chains, added to a mixture of crude oil, Corexit, and water, and then thoroughly mixed. The aggregation of the crude oil was monitored over time and compared to a control sample without HMCs. Corexit was found to be effective at breaking up the oil into small droplets, but was not effective at preventing droplet re-coalescence; after only 10 minutes of settling time, nearly all of the oil had coalesced into a single aggregate. Addition of HMCs to the system prevented aggregation for the duration of their study (30 minutes). Venkataraman and coworkers speculated that HMCs anchored into the oil droplets via the hydrophobic modification chains, leaving the chitosan to wrap around the exterior of the
droplets, therefore preventing oil re-coalescence through a combination of electrostatic and steric repulsion\(^1\).

The role of oil stabilizers is very similar to that of copolymer compatibilizers; both are used to prevent separation of immiscible phases. From a modeling standpoint, the HMCs can be thought of as comb copolymers, with chitosan being the comb backbone and alkane chains being the comb “teeth”. A number of studies have compared the behavior of comb copolymers to linear copolymers at the interface between immiscible polymer blends\(^{38-40}\). Lyatskaya et al. compared the behavior of comb and diblock copolymers, and concluded that long comb copolymers with multiple teeth more effectively compatibilize an interface than short diblocks\(^{39}\). Gersappe et. al. determined that combs with fewer long teeth located an interface more effectively than combs with many short teeth, and that comb copolymers oriented themselves at an interface more readily than multiblock copolymers of similar composition\(^{40}\). Numerous studies have focused on the role played by comb copolymer architecture in compatibilizing an interface\(^{41-43}\). Israels et al. showed that increasing the number and length of the “teeth” in a comb copolymer resulted in a reduction in the interfacial tension between two immiscible phases\(^{42}\). Potemkin et al. concluded that comb copolymers with long side chains undergo spontaneous bending of the backbone at an interface; this effect was significantly less pronounced as the side chain length was decreased\(^{43}\). Simulation studies have been used to look at how the architecture of comb copolymers affects their intramolecular interactions and conformation. Vasilevskaya et. al. showed that increasing the backbone length of a comb copolymer with attractive side chains leads to an increased number of intramolecular hydrophobic domains at a set grafting
density\textsuperscript{44}. Other simulation studies have analyzed the orientation of copolymers at the interface between two immiscible phases and their ability to prevent phase separation\textsuperscript{45-47}, but focused mainly on linear copolymers.

The goal of our research is to understand how the architecture of an HMC affects its ability to prevent oil aggregation with a view towards using HMCs for oil spill remediation. Simulations provide us with a molecular-level perspective on the interaction between HMCs and oil that cannot be obtained through experiment alone. Although the experimental work of Venkataraman and coworkers on HMCs has given good insight into the ability of HMCs to prevent oil coalescence, a number of questions remain such as the following. How does the length of the hydrophobic modification chains or the number of modification chains along the chitosan backbone (modification density) affect the efficacy of HMCs as oil stabilizers? What concentration of HMCs is necessary for oil droplet stabilization? What are the mechanisms by which HMCs stabilize oil droplets and how are they affected by the HMC architecture? What role does the chitosan backbone play in preventing oil droplets from aggregating? Answering these questions could lead to optimum HMC architectures for different types of oil spills and lower demand for oil dispersants.

In this paper we present the results of discontinuous molecular dynamics (DMD) simulations designed to determine how different HMC architectures and concentrations affect their ability to prevent oil aggregation. DMD is a fast alternative to traditional molecular dynamics (MD) that allows simulations of large systems of molecules at long time scales\textsuperscript{20}. The system contains two species: HMCs modeled as comb copolymers with a polar chitosan backbone and hydrophobic “teeth”, and oil molecules modeled as short linear
chains. Both the oil and chitosan molecules consist of chains of square-well and square-shoulder spheres. Water is modeled implicitly in all simulations, making the square-well and square-shoulder interactions potentials of mean force. Simulations are performed at various values of the modification chain length, modification density (percentage of chitosan backbone spheres containing modification chains), and overall HMC concentration. To determine how these parameters affect the HMCs ability to prevent oil aggregation, and the mechanism by which this is effected, we monitor the oil’s solvent accessible surface area (SASA), and the number, size, and shape of oil aggregates over the course of the simulations. The oil’s SASA gives a measure of how much oil surface area would be exposed to ocean bacteria leading to natural degradation. The oil aggregate size distribution indicates if the HMCs are preventing the oil from coalescing, therefore acting as an effective stabilizer. The shape of the oil aggregates is determined by calculating their asphericity, where the more aspherical the oil aggregates become, the more surface area is exposed to bacteria.

Highlights of our results are the following. Simulations started from a random initial configuration of HMC and oil chains lead to the formation of oil aggregates which are stabilized by HMCs. In systems containing HMCs with lower modification densities, the chitosan backbones form a network with each other and the modification chains penetrate into the oil aggregates, holding them in place and decreasing the rate of aggregation. In systems containing HMCs with higher modification densities, the modification chains form both intra-chain and inter-chain hydrophobic clusters and penetrate into oil aggregates while the chitosan backbones remain on the exterior of the aggregates. HMCs with a few long modification chains were slightly more effective at preventing oil coalescence than HMCs
with many short modification chains, even with the same total number of modification spheres. Finally, analysis of the contributions of the interaction energies between different types of spheres showed that the attractive interaction between the modification chains and the oil chains was the dominant interaction governing the HMC behavior, and that excluded volume interactions were not important in determining the effectiveness of HMCs even at high HMC volume fractions.

2.2 Methods

All species in the system are modeled as flexible chains of spheres. Oil molecules are modeled as linear 5-sphere chains where each sphere represents 3 carbons. We represented the oil with C15 alkanes based on an EPA study showing that these are one of the most common alkanes found in crude oil samples. HMCs are modeled as comb copolymers with a 50-sphere chitosan backbone and modification chain lengths ranging from 5 to 15 spheres, where each modification sphere represents 3 carbon atoms and their corresponding hydrogen atoms (consistent with our alkane coarse-graining), and each chitosan sphere represents a glucosamine monomer. The length of the chitosan chain was chosen to be 50 spheres, making it significantly longer than the alkane chains but not so long that we would have to simulate extremely large systems. We should point out that the chitosan backbone length in the experiments of Venkataraman and coworkers was much longer (300-1200 monomers), however their crude oil samples also contained significantly larger hydrocarbons than C15 alkanes. The modification density, which is the percentage of chitosan backbone spheres
containing modification chains, was 4%, 12%, or 20%. Diagrams illustrating the architecture of the HMCs used in the simulations can be seen in Figure 2.1. The sphere diameter, $\sigma$, for all species in the system is 1.0, and the bond length between adjacent spheres on the same chain is allowed to fluctuate between $\sigma(1+\delta)$ and $\sigma(1-\delta)$, where $\delta = 0.01$, making the spheres tangent. If adjacent spheres reach the maximum or minimum bond length, they hit an infinite repulsive potential that bumps them back to within the allowed bond distance$^{49,50}$. Stiffness is incorporated into these chains using “pseudobonds” in which the minimum and maximum angles that can form between next nearest neighboring spheres along the chain are set to 122 and 180 degrees respectively for the chitosan backbone, and 90 and 180 degrees respectively for the modification and oil chains. The chitosan backbone is stiffer than that of the alkane chains for two reasons: 1) to emulate the rigidity of a chitosan molecule, and 2) to mimic the electrostatic repulsion that occurs between the cationic chitosan monomers in slightly acidic conditions. The stiffness of the oil and modification chains is maintained by a set of pseudobonds to mimic the stiffness of real alkane chains, and to prevent the oil and modification chains from collapsing in an unrealistic manner.

Interactions between spheres are modeled using square-well or square-shoulder potentials depending on whether the interaction is attractive or repulsive. The potential energy between spheres of type i and j, $U_{ij}(r)$, as a function of distance between the spheres, $r$, is given by the following potentials:

$$U_{ij}(r) = \begin{cases} \infty & \text{if } r \leq \sigma \\ \epsilon_{ij} & \text{if } \sigma < r \leq \lambda \sigma \\ 0 & \text{if } r > \lambda \sigma \end{cases}$$  

(2.1)
where $\epsilon_{ij}$ is the interaction energy between spheres i and j, and the square-well or square-shoulder width is taken to be $\lambda \sigma = 1.75$. Table 1 lists the interaction energies between each type of sphere in the system. Negative values of the interaction energy represent attractions and positive values represent repulsions. The energies were chosen to mimic the types of interactions expected in a system of polar and non-polar molecules in water. Water is modeled implicitly in all simulations, making interaction energies between all spheres potentials of mean force in water. Chitosan, being polar, preferably interacts with itself over hydrocarbons. Therefore, the chitosan spheres are attracted to each other, but repelled by all other spheres in the system. The hydrophobic modification chains and the oil molecules represent alkanes, and therefore are attracted to each other and repelled by chitosan. The strength of attraction between chitosan spheres is half that of the attractive interactions between hydrophobic groups to help keep the chitosan backbone extended, as it would be in the presence of water molecules. The attractive energy between chitosan monomers is weaker than the attractive energy between modification spheres, and between oil spheres, so as to prevent the chitosan backbone from collapsing on itself, but is strong enough to encourage association with other chitosan molecules.

Details of the simulation conditions are the following. The packing fraction, $\eta = \frac{\pi N \sigma^3}{6V}$, is set to $\eta = 0.05$, where $N$ is the total number of spheres in the system, and $V$ is the volume of the simulation box. The packing fraction was kept low to emulate a dilute system of oil in water. The three system compositions considered in the simulations are HMC volume fractions ($\phi_{hmc}$) of 0.5, 0.35, and 0.2, where $\phi_{hmc}$ is the total volume of the HMC spheres divided by the total volume of all the spheres in the system. Systems with $\phi_{hmc}$
= 0.5 contain approximately 6000 HMC spheres and 6000 oil spheres, systems with \( \phi_{\text{hmc}} = 0.35 \) contain approximately 4200 HMC spheres and 7800 oil spheres, and systems with \( \phi_{\text{hmc}} = 0.2 \) contain approximately 2400 HMC spheres and 9600 oil spheres.

DMD simulations were used in place of traditional molecular dynamics (MD) to increase the speed of the simulations while still capturing the fundamental behavior of the system\textsuperscript{19}. Unlike traditional MD simulations, which numerically solve Newton’s equations of motion every time step, DMD is an event driven technique\textsuperscript{20}. The DMD simulation algorithm searches for the soonest-to-occur event (collision between spheres), advances the time to the point that the collision occurs, and analytically calculates the new particle positions and velocities after the collision\textsuperscript{20}. An event occurs any time a particle reaches a discontinuity in the interaction potential as a function of separation distance such as at the boundary of a hard sphere, square-well, or square-shoulder potential. Because the method is event driven, a collision occurs every time step, avoiding the need to re-calculate collision dynamics multiple times before particles are within the interaction distance of each other. Following a collision, the particles in the simulation move linearly until their next collision occurs.

The simulations began with a random initial configuration of oil and HMCs. The temperature of the system was controlled using an Andersen thermostat, where the average system reduced temperature is \( T^* = k_b T/|\epsilon_{12}| \), where \( \epsilon_{12} \) is the interaction energy between spheres of type 1 and type 2 (type refers to whether the sphere is a chitosan sphere, modification sphere, or oil sphere). In the Andersen thermostat the temperature is regulated using “ghost collisions” where a sphere in the system is randomly chosen to collide with a
“ghost” sphere to maintain the Maxwell-Boltzmann velocity distribution around the desired temperature$^{51}$. The first 150 million collisions of the simulation were run at a high temperature of $T^* = 8.0$ to equilibrate the system, and remove any artifacts that might arise from the choice of the initial configuration. Following the initial high-temperature portion of the simulation, the temperature was dropped by $T^* = 0.1$ every million collisions until a temperature of $T^* = 5.0$ was reached. Following this cooling, an even slower cooling procedure was performed, lowering the temperature by $T^* = 0.01$ every million collisions until the desired temperature of $T^* = 1.3$ was achieved. The rate of cooling was slowed as the set temperature was approached to allow gradual aggregation of the oil molecules. A gradual cooling procedure also more accurately represent reality, as the temperature of a system cannot suddenly drop without passing through intermediate temperatures. The temperature was held constant at $T^* = 1.3$ for the duration of the simulation (up to 2 billion collisions). A set temperature of $T^* = 1.3$ was used because it was the highest temperature at which oil molecules aggregated using the interaction energies discussed previously. At a temperature of $T^* = 1.2$ aggregation occurred rapidly and was not a gradual process over time, while at a temperature of $T^* = 1.4$ minimal aggregation was observed by the end of the simulation. We chose a temperature that allowed the oil aggregation to occur over the course of hundreds of millions of collisions because we felt that this allowed the HMCs to have sufficient time to interact with the oil. Another way to equilibrate the system would have been to only include the oil molecules in the high temperature stage, and add chitosan after the set temperature was reached. However this might have made it harder for the chitosan to efficiently break out of its initial configuration at low temperatures. It might have also led to
the formation of different equilibrium structures because oil aggregation would have already begun by the time HMCs were added. We plan to investigate this phenomenon in future work to determine how the addition of HMCs at different times affects the system equilibrium, giving insight into the importance of response time to HMC efficacy.

The efficacy of the HMCs as oil stabilizers was measured in a number of ways. First, the oil’s solvent accessible surface area (SASA) was measured using a built-in SASA function in the Visual Molecular Dynamics (VMD) software throughout the course of the simulation to observe the overall extent of aggregation. The SASA includes: (1) the area on the exterior of oil aggregates (which would be in contact with water if there had been explicit solvent) and (2) the area on the exterior of the oil aggregates that is in contact with those modification chains that lie on the exterior of the oil aggregates. The SASA was measured by removing all HMCs from the simulation box, and rolling a probe sphere of diameter 1.4 around the surface of the oil molecules. The probe sphere was chosen to be larger than the oil sphere diameter to prevent voids in the middle of aggregates (caused by removing HMC) from significantly adding to the SASA. To confirm that we selected an appropriate probe sphere size, we measured the oil SASA without removing the modification spheres present in the middle of oil aggregates for systems with the highest concentration of modification spheres. Doing this eliminated the possibility of artificial pores being present in the middle of the aggregates. The oil SASA only decreased by 4%, showing that our choice of probe size eliminated nearly all SASA contributions from the interior of the oil aggregates.
Second, we calculated the percent increase in oil SASA for various HMC architectures over systems without HMCs present. The percent increase in the oil SASA is given by:

\[
\% \text{ increase in oil SASA} = \left( \frac{\text{SASA}_{\text{HMC}} - \text{SASA}_{\text{no HMC}}}{\text{SASA}_{\text{no HMC}}} \right) \times 100
\]  

(2.2)

where SASA_{HMC} is the oil SASA with HMCs present and SASA_{no HMC} is the oil SASA without HMCs present. The number and size of the oil aggregates was also calculated at various time points to determine if the HMCs could prevent aggregation and to give insight into the mechanism of aggregation. These quantities measure how effectively the HMCs prevent oil aggregates from coalescing over time.

Third, we analyzed the shape of the oil aggregates to determine how the HMC architecture affected the ability of oil to form small droplets. The aggregate shape was characterized in terms of its asphericity.\(^{52,53}\) This method involves calculating the principal components of the gyration tensor, \(\mathbf{S}\), for each oil aggregate:

\[
\mathbf{S} = \frac{1}{N} \left( \begin{array}{ccc}
\sum_i (x_i - x_{cm})^2 & \sum_i (x_i - x_{cm})(y_i - y_{cm}) & \sum_i (x_i - x_{cm})(z_i - z_{cm}) \\
\sum_i (x_i - x_{cm})(y_i - y_{cm}) & \sum_i (y_i - y_{cm})^2 & \sum_i (y_i - y_{cm})(z_i - z_{cm}) \\
\sum_i (x_i - x_{cm})(z_i - z_{cm}) & \sum_i (y_i - y_{cm})(z_i - z_{cm}) & \sum_i (z_i - z_{cm})^2
\end{array} \right)
\]  

(2.3)

where \(x_i, y_i,\) and \(z_i\) are the coordinates of each oil sphere, \(i\), in the aggregate, \(x_{cm}, y_{cm},\) and \(z_{cm}\) are the coordinates of the aggregate’s center of mass, and \(N\) is the total number of oil spheres in the aggregate. The eigenvalues of the gyration tensor \(\lambda_1, \lambda_2,\) and \(\lambda_3,\) are the principal components of the radius of gyration, \(R_g\), defined by:

\[
R_g^2 = \lambda_1 + \lambda_2 + \lambda_3
\]  

(2.4)
where $\lambda_1$, $\lambda_2$, and $\lambda_3$ are the eigenvalues of the gyration tensor. These principal components are then used to calculate the oil aggregate asphericity, $A$, defined as:

$$A = \frac{(\lambda_1-\lambda_3)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_2)^2}{2(\lambda_1+\lambda_2+\lambda_3)^2} \quad (2.5)$$

where $\lambda_1 \geq \lambda_2 \geq \lambda_3$. The asphericity has a minimum value of 0 for a perfectly spherical aggregates and a maximum value of 1 for completely linear aggregates. The radius of gyration and asphericity data were averaged over the final 100 million collisions of the simulations (with data recorded every 10 million collisions) and over 5 simulation replicates. All of the previously mentioned analysis techniques were performed on oil aggregates in systems with and without HMCs to determine the impact of HMCs on oil aggregation. Error bars for all quantities calculated represent the standard deviation from the average of each data point over 3 to 5 replicates.

### 2.3 Results

Snapshots of the intermediate and final configurations in a simulation of 4% modified HMCs with 15-sphere modification chains are compared to snapshots at the same time points in a simulation containing oil only in Figure 2.1. Figures 2 (a) and (b) show system configurations with and without HMCs at 100 million collisions during the initial high temperature ($T^* = 8.0$) portion of the simulations when the system is randomized. Figures 2 (c) and (d) show both systems at 750 million collisions after the set temperature of $T^* = 1.3$ has been reached. At this point aggregation had occurred in each system, however the system without HMCs aggregated significantly more than the system with HMCs. At the
end of the simulations (2 billion collisions), the oil-only system formed a single large aggregate as can be seen in Figure 2.1(e). Note that although there appear to be four separate aggregates in Figure 2.1(e), there is actually only one aggregate, which crosses the periodic boundary conditions. In contrast, the oil + HMC system formed many smaller oil aggregates, which can be seen in Figure 2.1(f). We speculate that the HMCs help stabilize the smaller oil aggregates and prevent them from coalescing via the modification chains anchoring into the oil droplets and the chitosan backbone remaining on the perimeter of each droplet. We believe that a combination of repulsion between the chitosan and oil spheres and steric hindrance of the chitosan backbone covering the oil aggregates prevents the oil from coalescing as much as in systems without HMCs. Once the modification chains of the HMCs penetrate the oil aggregates, the motion of the chitosan backbone is restricted because it is pinned to the oil aggregates at multiple locations. Therefore, the backbone acts to block new oil chains from joining the aggregate.

The oil surface area over time was calculated to determine the efficacy of the HMCs in preventing oil aggregation; larger oil surface areas mean less aggregation. The purpose of using HMCs is to maximize the oil surface area over time, therefore providing more surface area for hydrocarbon-degrading bacteria to naturally degrade the oil before it forms a surface slick. Figure 2.3 shows the oil SASA over time at $\phi_{\text{hmc}} = 0.5$ for HMCs with (a) 5-sphere and (b) 15-sphere modification chains and various modification densities. The green line in both plots shows the oil SASA over time without any HMCs in the system, while the pink line shows the oil SASA in a system with unmodified chitosan. The black, red, and blue lines show the oil SASA over time in systems of HMCs that are 4%, 12%, and 20% modified.
respectively. It can be seen that the oil SASA decreases over time in systems with and without HMCs because small oil aggregates coalesce into larger ones. Increasing the modification density increases the oil SASA at the end of the simulations at all modification densities tested for HMCs with 5-sphere modification chains. A somewhat different trend occurs for HMCs with 15-sphere modification chains. The oil SASA increases with increasing modification density from 4% to 12% but reaches a plateau thereafter; the oil SASA is nearly identical at modification densities of 12% and 20%. One possible explanation for the plateau is that the total HMC concentration is so high that it causes the oil droplets to be saturated with HMC molecules. To test this hypothesis, we performed simulations at lower HMC concentrations of $\phi_{\text{hmc}} = 0.35$ and $\phi_{\text{hmc}} = 0.2$. The results from these simulations will be discussed in the next paragraph. The conclusion that we draw from Figure 2.3 is that HMCs with long modification chains are more effective at lower modification densities than HMCs with short modification chains. We speculate that the longer modification chains not only penetrate more deeply into the oil aggregates, but also have a higher probability of interacting with more oil chains before they have the chance to aggregate due to their extended “reach”. The increased interaction between long modification chains and oil disrupts oil chain packing, therefore making it more difficult for oil chains to form spherical droplets with minimum surface area. To ensure that 2 billion collisions was enough to capture the majority of the aggregation behavior, we also ran some sample simulations for 4 billion collisions and determined that the oil SASA decreased by less than 5% between 2 and 4 billion collisions. Therefore we ended our simulations at 2 billion collisions.
Figure 2.4 summarizes the efficacy of different HMC architectures at concentrations, \( \phi_{\text{hmc}} = 0.5, 0.35, \) and 0.2, by plotting the percent increase in oil SASA resulting from (a) 5-sphere and (b) 15-sphere modification chain HMCs over the oil SASA without HMCs present at the end of the simulations at various modification densities. Figure 2.4 confirms that there is a relatively linear trend in the percent increase in oil SASA with increasing modification density for HMCs with 5-sphere modification chains. In contrast, as we saw from Figure 2.3, there is clearly a modification density of HMCs with 15-sphere modification chains, above which the percent increase in oil surface area remains essentially unchanged, even at the lowest HMC concentration tested (\( \phi_{\text{hmc}} = 0.2 \)). This shows that the saturation phenomenon persists regardless of the HMC concentration, and we can therefore conclude that it is unnecessary to use a modification density above 12% for HMCs with 15-sphere modification chains at any HMC concentration. To determine the optimum modification density with 5-sphere modification chains, we would need to increase the modification density further, however this would far exceed the modification densities used in experiments.

Oil aggregate size distributions were calculated to quantify the number of oil aggregates of each size at different times throughout the simulation. These distributions were averaged over time and over 5 simulation replicates. Data reported at 0.75, 1.25, and 2 billion collisions was averaged from 0.7-0.8, 1.2-1.3, and 1.9-2.0 billion collisions respectively (with data collected every 0.01 billion collisions). Oil chains were defined to be in a cluster if any spheres on two separate chains were within each other’s square-wells. If an oil cluster contained 5 or more oil chains, it was considered an aggregate. Figure 2.5
compares the oil aggregate size distributions for simulations of 20% modified HMCs with 5 or 15-sphere modification chains at $\phi_{hmc} = 0.5$ at various times throughout the simulation. The number of small aggregates (50 chains or less) significantly decreases over time as the aggregates coalesce. This behavior reflects what happens in an oil spill scenario, where small oil droplets coalesce into a slick on the ocean surface. Figure 2.5 also shows that the aggregate size distributions using 5 or 15-sphere modification chains differ significantly for 20% modified HMCs. There are nearly twice as many small oil aggregates (50 chains or less) for HMCs with 5-sphere modification chains as there are for 15-sphere modification chains after 1.25 billion collisions. By the end of the simulations, the aggregate size distribution is skewed to favor smaller aggregates for 5-sphere modification chains, but is more evenly distributed for 15-sphere modification chains. Therefore, at this high modification density, HMCs with short modification chains result in many small aggregates while HMCs with long modification chains result in a few large oil aggregates.

Oil aggregate size distributions were also calculated for the other $\phi_{hmc}$ to determine if the trends just described also hold in these cases. Figure 2.6 shows the oil aggregate size distributions at the end of simulations of 20% modified HMCs with 5 or 15-sphere modification chains at $\phi_{hmc} = 0.5$, 0.35, and 0.2. (Note that unlike Figure 2.5, these distributions are all calculated at the end of simulations and not at different time points throughout the simulations.) The aggregate size distributions at $\phi_{hmc} = 0.5$ and 0.35 show similar behavior to that seen in Figure 2.5, where short modification chains lead to more small oil aggregates and long modification chains lead to more large oil aggregates. At $\phi_{hmc} = 0.2$ there is no distinguishable difference in the oil aggregate size distribution between
HMCs with short or long modification chains because there were too few HMCs to make a significant impact on the oil aggregate size distribution. However, even low concentrations of HMCs lead to more small aggregates than systems without HMCs, which form a single large aggregate by the end of the simulations. Therefore, the HMCs are beneficial in reducing oil aggregation even at the lowest concentrations tested.

In addition to influencing the size distribution of oil aggregates, penetration of the hydrophobic modification chains affects the shape of the oil aggregates. Figure 2.7 compares snapshots of the system at the end of simulations using 20% modified HMCs with 5 and 15-sphere modification chains with those of a system containing oil only to get a qualitative perspective on the shape of the oil aggregates in each system. Figure 2.7(a) shows a system containing oil only, while Figures 7(b) and 7(c) show systems with 5 and 15-sphere modification chain HMCs respectively at $\phi_{\text{hmc}} = 0.5$. The HMCs are removed from the snapshots to more clearly see the oil aggregates. The oil chains that are not in aggregates are in yellow, and the largest oil aggregates in the system are in different colors. Note that the different colors are used only to distinguish between unique aggregates and are no indication of size or geometry of the aggregates. The system without HMCs forms one large aggregate that includes nearly all of the oil chains in the system. The oil aggregates in the system containing HMCs with 5-sphere modification chains, are relatively symmetrical and tightly packed. In contrast, the oil aggregates in the system containing HMCs with 15-sphere modification chains are asymmetrical and stretched in one or more directions, helping to increase the oil SASA. Note that although the green aggregate in the snapshot of the system containing 15-sphere modification chains appears spherical, it is significantly stretched in the
direction going into the page. The asymmetry in oil aggregate shape is most noticeable at high modification densities, because there are more modification chains present to penetrate each oil droplet. Figure 2.7(c) reveals many pores in the oil aggregates, providing visual evidence of the ability of long modification chains to penetrate into the aggregates. In contrast, very few pores are visible in the oil aggregates in Figure 2.7(b), showing the inability of the short modification chains to penetrate deeply into the oil aggregates. We would like to clarify that the pores observed in Figure 2.7(c) are merely the voids left by removing the HMCs from the snapshot. Their presence demonstrates how effectively the long modification chains penetrate the oil aggregates.

Oil aggregate asphericity was calculated to get a more quantitative measure of the shape of oil aggregates. Asphericity gives more insight into the shape of aggregates than the radius of gyration because it shows if the aggregate is elongated in any direction. Table 2 shows the average values of asphericity for oil aggregates in systems of HMCs with various modification densities and modification chain lengths at $\phi_{hmc} = 0.5$. There is little variation in oil aggregate asphericity with modification density for 5-sphere modification chains, but there is a noticeable variation with modification density for 15-sphere modification chains. Increasing the modification density from 4% to 20% for 15-sphere modification chains increases the asphericity by approximately 50%, meaning that the oil aggregates become more elongated. Not only does this confirm the behavior observed in Figure 2.7, but it also provides an explanation for why HMCs with 5-sphere modification chains lead to a greater number of small aggregates, but still result in a lower oil surface area. As oil chains coalesce, spherical droplets form to maximize the number of favorable oil-oil contacts,
resulting in the minimum possible surface area. Therefore, even though there are a greater number of small aggregates with 5-sphere modification chains, all of the aggregates minimize their surface area by taking on a spherical shape. Although there are fewer (and larger) aggregates in systems with 15-sphere modification chains, the HMCs more effectively penetrate into the oil aggregates, elongating the aggregates, and creating more oil surface area.

To prove that long modification chains more effectively penetrate into the oil aggregates, we calculated the percentage of modification spheres interacting with oil spheres in systems of HMCs with 5 and 15-sphere modification chains. The percentage of modification spheres interacting with oil spheres over time for 20% modified HMCs at $\phi_{hmc} = 0.5$ can be seen in Figure 2.8. Two spheres are considered to be interacting if they are within each other’s square-wells, therefore experiencing an attractive interaction for each other. Snapshots of systems with 15-sphere modification chain HMCs (top) and 5-sphere modification chain HMCs (bottom) at the end of simulations are also shown to illustrate the difference in the interaction of short and long modification chains with oil aggregates. Figure 2.8 shows that there is a drastic difference in the percentage of modification spheres interacting with oil spheres for the two different modification chain lengths. By the end of the simulations approximately 80% of the modification spheres interact with oil spheres for HMCs with 15-sphere modification chains while only about 30% interact with oil for HMCs with 5-sphere modification chains. The increased ability of long modification chains to penetrate oil aggregates is illustrated in the snapshots to the right of the chart in Figure 2.8. The top snapshot (15-sphere modification chains) shows good mixing between the
modification spheres and oil spheres, while the bottom snapshot (5-sphere modification chains) shows separate oil and modification domains with no significant penetration of the modification chains into the oil aggregates. The percentage of modification spheres interacting with oil spheres is nearly identical regardless of modification density (data not shown).

Modification chain length and modification density not only affect HMCs’ interactions with oil, but also affect HMCs’ interaction with each other. Figure 2.9 shows snapshots of systems of 20% modified HMCs at the end of simulations with 5 and 15-sphere modification chains at $\phi_{hmc} = 0.2$, as well as a snapshot at the end of a simulation using unmodified chitosan. (Note that oil was present in each of these simulations, but has been removed to allow easier visualization of the HMC network). Each system contains the same total number of HMC spheres (or unmodified chitosan spheres in Figure 2.9(a)). The snapshots in Figure 2.9 show that varying the modification chain lengths results in different HMC network architectures. The longer “reach” of the 15-sphere modification chains causes the modification chains to dominate network formation, as indicated by the large hydrophobic clusters in red. The shorter “reach” of the 5-sphere modification chains causes the chitosan backbone to dominate network formation, meaning that the orientation of the molecules within the network is more controlled by the chitosan backbone stiffness than by the hydrophobic interaction between modification chains. The network formed by the unmodified chitosan is also controlled by chitosan stiffness due to the lack of hydrophobic interactions by modification chains. The strong association of long modification chains shows how long modification chain HMCs lead to more elongated oil aggregates.
At this point the effectiveness of HMCs as oil stabilizers has been analyzed using a number of techniques, however one important question remains. Are HMCs with few long modification chains better at preventing oil aggregation than HMCs with many short modification chains if the total number of modification spheres is the same? To determine if the architecture plays a role in oil stabilization, it is necessary to compare two different HMC architectures with the same total number of chitosan and modification spheres per molecule. This determines if stabilizing oil droplets depends on more than just the total number of modification spheres in the system. Therefore we compared simulations of 4% modified HMCs with 15-sphere modification chains and 12% modified HMCs with 5-sphere modification chains in oil at $\phi_{\text{hmc}} = 0.5$. The HMCs that were 4% modified with 15-sphere modification chains had two modification chains on the chitosan backbone, while the HMCs that were 12% modified with 5-sphere modification chains had six modification chains on the chitosan backbone. However, both HMCs had the same total number of chitosan spheres (50) and modification spheres (30) per molecule. The final oil SASA using HMCs with 15-sphere modification chains was 12% higher than the oil SASA using HMCs with 5-sphere modification chains (data not shown). This is consistent with the behavior discussed previously where longer modification chains led to a larger oil SASA, and also shows that the HMC architecture does play a role in oil dispersion, even for HMCs with the same number of modification spheres.

The oil aggregate size distribution for these two HMC architectures was calculated to compare the number and size of oil aggregates formed. This can be seen in Figure 2.10, which shows that HMCs with long modification chains result in fewer small aggregates ($<50$
chains) than HMCs with short modification chains even though the total number of spheres for the two HMCs is the same. We also observed the formation of slightly more large aggregates (>200 chains) with long modification chain HMCs than with short modification chain HMCs. These results are in agreement with the previously mentioned aggregate size distributions, where long modification chains promoted the formation of larger oil aggregates. From these results we can conclude that HMC architecture does affect both the oil SASA and the size distribution of oil aggregates, and that the HMC efficacy is dependent on more than just the total number of modification spheres in the system.

To ensure that the increase of oil SASA in systems containing HMCs was not simply due to the HMCs “getting in the way” of the oil chains, the interaction energies between the various species were systematically set to zero one at a time, to pinpoint the important interactions. The rationale for this exercise is that if the HMCs simply prevented oil aggregation because they took up space in the simulation box, setting attractive/repulsive interactions between HMCs and oil to zero would result in approximately the same oil SASA over time as simulations with all of the interactions accounted for between the HMCs and oil. Note that the attractive interaction between oil spheres is always present to ensure that the oil aggregates in all simulations. Four total cases were tested to determine the most important interaction energies: (1) no interactions were set to zero, (2) chitosan backbone interactions were set to zero, (3) modification chain interactions were set to zero, (4) all interaction energies were set to zero.

Table 3 shows the percent increases in oil SASA over systems of oil only (defined in Equation 2) for the four cases described above. This data confirms that the interaction
between modification chains and oil chains controls the ability of HMCs to prevent oil aggregation because there is only a slight difference in the oil SASA between case 2 systems (backbone interactions set to zero) and the case 1 systems (no interactions set to zero) for both 5-sphere and 15-sphere modification chains. It is somewhat surprising to find that the modification chain interactions are the most important interactions for HMCs with 5-sphere modification chains, especially after determining that only about 30% of the modification spheres interact with the oil. However, this supports the argument that the modification chains act to anchor the oil droplets to the chitosan backbone, therefore restricting their movement and preventing aggregation even though they cannot deeply penetrate the oil droplets. Comparison of case 3 systems (modification interactions set to zero) and case 1 systems (no interaction energies set to zero) shows that the chitosan backbone interactions play only a minor role in preventing oil aggregation because the increase in oil SASA drastically decreases when only backbone interactions are present. The backbone interactions most likely play a minor role because our simulations use an implicit solvent model; therefore there are no polar water molecules for the chitosan spheres to be attracted to. We also showed that the efficacy of the HMC stabilizers is not purely dependent on the excluded volume interactions between the HMCs and oil. In fact, excluded volume interactions alone play a minor role in the HMC’s ability to prevent oil aggregation because the oil SASA at the end of case 4 simulations (all interaction energies set to zero) is only 10-12% larger than in systems containing oil only.
2.4 Discussion and Conclusions

We have presented the results of discontinuous molecular dynamics simulations aimed at determining the efficacy of HMCs as oil stabilizers, and the key parameters that govern their ability to prevent oil aggregation. HMCs with modification chain lengths of 5 and 15 spheres, and modification densities of 4%, 12% and 20% were considered. Throughout the course of the simulations the extent of oil aggregation was monitored in a number of ways for systems with different HMC architectures. First we monitored the oil SASA throughout the course of simulations to get a “big picture” look at the effectiveness of the HMCs, where larger oil SASAs indicate more effective HMCs. This analysis determined if the HMCs were effective or not, but did not give insight into how they prevented oil aggregation. We concluded that all HMCs tested led to larger oil SASA over time than systems without HMCs, for systems starting in a random initial configuration of oil and HMC molecules in the simulation box. Additionally, increasing the modification chain length and modification density led to an increase in the oil SASA. There did appear to be a saturation modification density above which there was no increase in oil SASA for the 15-sphere modification chains, as the HMCs that were 12% and 20% modified had nearly identical oil SASA. This shows that it is unnecessary to exceed a modification density of 12% when using the 15-sphere modification chains. However, there was an increase in oil SASA with increasing modification density for HMCs with 5-sphere modification chains for all modification densities tested.
Next we looked at the oil aggregate size distributions for systems of HMCs with various modification densities and modification chain lengths. This analysis revealed that HMCs with long modification chains led to fewer and larger oil aggregates than systems with short modification chains. This contradicted the results we expected, given that HMCs with long modification chains had larger oil SASAs than HMCs with short modification chains. One would have expected that an increase in oil SASA would coincide with a greater number of small oil aggregates rather than fewer large oil aggregates. The only way it would have been possible to have a larger oil surface area and larger oil aggregates would be for the aggregates to be stretched. To test this hypothesis we first looked at snapshots of the oil phase at the end of simulations to get a qualitative understanding of the oil aggregate shape. It was clear from these snapshots that the oil aggregates in systems of HMCs with long modifications were indeed more stretched and aspherical than oil aggregates in a system with short modification chain HMCs. We concluded that the oil aggregate asphericity for HMCs with 5-sphere modification chains does not vary significantly with increasing modification density, while that for 15-sphere modification chains increases by approximately 50% going from the lowest to highest modification densities. The longer modification chains more effectively penetrate into the oil aggregates, deforming their shape.

Finally we determined that the interaction between the modification chains and the oil chains was the most important interaction in the HMCs preventing oil aggregation. The oil SASA in a system containing HMCs with only modification chain interactions was very similar to that of a system with all HMC interactions. We believe that short modification chain HMCs prevent oil aggregation by anchoring the chitosan backbones to the oil
aggregates, in turn restricting the movement of the aggregates and preventing aggregation. In contrast, we have shown that the long modification chain HMCs prevent aggregation by penetrating deeply into the aggregates and preventing them from forming small, spherical droplets.

In conclusion we have gained insight into the optimum HMC architectures for preventing oil aggregation. Our results conceptually agree with the experimental work of Venkataraman and coworkers\(^1\) and can be used to help design effective HMC oil stabilizers.

One way that our simulations differ from the work of Venkataraman and coworkers is that we did not include Corexit in the system. Corexit is primarily used to break up oil into smaller droplets. In a way we accounted implicitly for addition of Corexit by starting our simulations in a random initial configuration with oil molecules already broken up. If we were to include Corexit in the simulations, the efficacy of HMCs might increase due to electrostatic attraction between the cationic chitosan monomers and the anionic surfactant, dioctyl sodium sulfosuccinate (DOSS), present in Corexit. This might allow the HMCs to adhere more strongly to the oil droplets and possibly travel to the oil/water interface more quickly. We plan to include Corexit in a follow-up study.

In its current state our model can be easily applied to study different chitosan molecular weights, different types of modification chains, and different HMC concentrations. We are currently extending our model to include more details about the chitosan backbone such as the degree of acetylation and surface charge, making the model more universally applicable to a variety of conditions.

Although our model of an oil/HMC system has many advantages for qualitative study of HMCs as oil dispersant additives, it has several limitations. The first limitation is that we
used an implicit solvent approach, which does not accurately represent the diffusion of molecules or hydrodynamics. Because there are no water molecules to collide with the solute, the solute molecules travel more quickly through the simulation box than they would in the presence of water. The second limitation is that it is difficult to make a direct correlation between the reduced temperature in the simulation and real temperature. Values of the reduced temperature and interaction energies were chosen to make the oil aggregate gradually over the course of the simulation (several hundred million collisions) as opposed to being derived via multi-scale modeling of atomistic simulations. Therefore the chosen temperature is not directly related to the real temperature, as would be the case in atomistic simulations. The third limitation of this method is that we do not have a way to correlate simulation time to real time because molecules move in a straight line in a vacuum between collisions, rather than traveling through solvent. In order correlate our results to a real time scale we would have to compare the time it takes for a specific molecular mechanism to occur in experiments and simulations. Unfortunately we are unable to identify a mechanism that would be distinguishable in both experiments and simulations to compare the time scales.

2.5 Acknowledgements

This work was supported by the GAANN Computational Science Fellowship and the Gulf of Mexico Research Initiative (GOMRI). This work was also supported in part by the NSF’s Research Triangle MRSEC, DMR-1121107.
2.6 References


Figure 2.1. Schematic diagram showing the different architecture HMCs used in simulations of systems containing HMCs and oil.
Figure 2.2. Snapshots of oil aggregation during simulations of systems containing oil only and oil + 4% modified HMCs with 15-sphere modification chains where $\phi_{\text{hmc}} = 0.5$. Chitosan spheres are blue, modification spheres are red, and oil spheres are yellow.
Figure 2.3. Effect of HMC modification density on ability of HMCs with (a) 5 and (b) 15-sphere modification chains to prevent oil aggregation for $\phi_{hmc} = 0.5$. 
Figure 2.4. Percent increase in oil SASA for HMCs with (a) 5 or (b) 15-sphere modification chains and varying modification density.
Figure 2.5. Oil aggregate size distributions over time using 20% modified HMCs with (a) 5 or (b) 15-sphere modification chains at $\phi_{\text{hmc}} = 0.5$. 
Figure 2.6. Oil aggregate size distributions at the end of simulations using 20% modified HMCs with 5 or 15-sphere modification chains with $\phi_{\text{hmc}} =$ (a) 0.5, (b) 0.35, and (c) 0.2
Figure 2.7. Snapshots of oil aggregates at the end of simulations containing (a) only oil, (b) 20% modified HMCs with 5-sphere modification chains, and (c) 20% modified HMCs with 15-sphere modification chains. Systems with HMCs were at $\phi_{hmc} = 0.5$. 

Figure 2.8. Percentage of modification spheres interacting with oil spheres during simulations with 20% modified HMCs with 5 and 15-sphere modification chains
Figure 2.9. Snapshots of networks formed by (a) unmodified chitosan, (b) 20% modified HMCs with 5-sphere modification chains, and (c) 20% modified HMCs with 15-sphere modification chains. Chitosan spheres are in blue and modification spheres are in red.
Figure 2.10. Oil aggregate size distributions comparing systems of HMCs with (a) few long modification chains and (b) many short modification chains at $\phi_{\text{hmc}} = 0.5$
Table 2.1. Interaction energies between each type of sphere

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<th>Chitosan</th>
<th>Modification</th>
<th>Oil</th>
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<td>Chitosan</td>
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<td>+0.5</td>
<td>+0.5</td>
</tr>
<tr>
<td>Modification</td>
<td>-0.5</td>
<td>-0.5</td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td></td>
<td></td>
<td>-0.5</td>
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Table 2.2. Average asphericity of oil aggregates for systems of HMCs with various modification densities and modification chain lengths for $\phi_{hmc} = 0.5$

<table>
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<th>Modification density</th>
<th>5-sphere modification chains</th>
<th>15-sphere modification chains</th>
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<tr>
<td>4%</td>
<td>$0.17 \pm 0.18$</td>
<td>$0.21 \pm 0.16$</td>
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<td>12%</td>
<td>$0.19 \pm 0.16$</td>
<td>$0.33 \pm 0.19$</td>
</tr>
<tr>
<td>20%</td>
<td>$0.18 \pm 0.14$</td>
<td>$0.33 \pm 0.17$</td>
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Table 2.3. Percent increase in oil SASA in systems of 4% modified HMCs with 5 and 15-sphere modification chains at $\phi_{hmc} = 0.5$ over systems of oil only

<table>
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<th>Case #</th>
<th>HMC interactions set to zero</th>
<th>5-sphere modification chains</th>
<th>15-sphere modification chains</th>
</tr>
</thead>
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<td>1</td>
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<td>35.3%</td>
<td>83.6%</td>
</tr>
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<td>2</td>
<td>Backbone</td>
<td>31.1%</td>
<td>75.3%</td>
</tr>
<tr>
<td>3</td>
<td>Modification</td>
<td>20.1%</td>
<td>18.2%</td>
</tr>
<tr>
<td>4</td>
<td>All</td>
<td>10.5%</td>
<td>12.1%</td>
</tr>
</tbody>
</table>
CHAPTER 3

Development of a Coarse-Grained Model of Chitosan for Predicting Solution Behavior

Chapter 3 is essentially a manuscript by Steven W Benner and Carol K Hall accepted by the Journal of Physical Chemistry B.
Development of a Coarse-Grained Model of Chitosan for Predicting Solution Behavior

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Abstract

A new coarse-grained model of chitosan has been developed for predicting solution behavior as a function of degree of acetylation (DA). A multi-scale modeling approach was used to derive the energetic and geometric parameters of this implicit-solvent, coarse-grained model, from all-atom simulations of chitosan and chitin molecules in explicit water. The model includes representations of both protonated D-glucosamine (GlcN\(^+\)) and N-acetyl-D-glucosamine (GlcNAc) monomers, where each monomer consists of three coarse-grained sites. Chitosan molecules of any molecular weight, DA, and monomer sequence can be built using this new coarse-grained model. Discontinuous molecular dynamics simulations (DMD) of chitosan solutions show increased self-assembly in solution with increasing DA and chitosan concentration. The chitosan solutions form larger percolated networks earlier in time as DA and concentration increase, indicating “gel-like” behavior, which qualitatively matches experimental studies of chitosan gel formation. Increasing DA also results in a greater number of monomer-monomer associations, which has been predicted experimentally based on an increase in the storage modulus of chitosan gels with increasing DA. Our model
also gives insight into how the monomer sequence affects self-assembly, and the frequency of interaction between different pairs of monomers.

### 3.1 Introduction

Chitosan is a versatile biopolymer derived from chitin, a component in the exoskeleton of crustaceans such as crabs, shrimp, insects, etc. It is a polysaccharide that consists of a random distribution of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) monomers, resulting from the deacetylation of chitin (which contains only GlcNAc monomers). The design of self-assembled systems based on chitosan has become an active field of research because chitosan is biocompatible, polycationic, and chemically modifiable as a result of its amine functional group. Chitosan has shown promise for use in applications ranging from wound healing\(^1\)-\(^3\) and drug delivery\(^4\)-\(^8\) to water purification\(^9\)-\(^11\) and oil spill remediation\(^12,\ 13\). Chitosan self-assembly in solution depends strongly on the degree of acetylation (DA) and pH\(^14\)-\(^18\). Increasing the DA and the solution pH causes the chitosan chains to associate and form gels due to increased hydrophobicity and decreased net charge. A tool that could quickly explore the effects of changes in DA and net charge on chitosan self-assembly would be valuable in understanding the solution behavior of chitosan under a wide variety of conditions. In this paper we develop a coarse-grained model of chitosan that can be used to simulate chitosan solutions of varying molecular weight, degree of acetylation, and monomer sequence to predict the conditions that lead to chitosan self-assembly.
Network formation of chitosan in solution is strongly dependent on the DA. Pure chitosan (DA = 0) contains only GlcN monomers, which are protonated in solution at a pH up to their pKa (~6.5), giving the monomers a net positive charge.\textsuperscript{18-21} We will refer to protonated GlcN as GlcN\textsuperscript{+}. These monomers repel each other in solution due to having like charges. When the DA is increased above zero, the polymer consists of a combination of GlcN\textsuperscript{+} and GlcNAc monomers. The GlcNAc monomers have no charge and associate in solution via a combination of hydrophobic and hydrogen bonding interactions.\textsuperscript{22} A chitin chain, which consists of only GlcNAc monomers, is insoluble due to the strong association between the monomers. Therefore at or below a pH below 6.5, the combination of GlcN\textsuperscript{+} and GlcNAc monomers leads to a balance between electrostatic repulsion and hydrophobic association of the chitosan chains.

Previous work suggests that at a pH below the pKa, chitosan exhibits different solution behavior in three ranges of DA: below 25%, between 25% and 50%, and above 50%.\textsuperscript{17, 19, 20} At DA’s below 25%, chitosan behaves as a polyelectrolyte with protonated amine groups; the chains in solution have minimal association due to the high charge density along the chain.\textsuperscript{17} At DA’s above 50%, chitosan behaves like a hydrophobic polymer with only isolated positive charges, leading to intra-molecular and intermolecular associations, and hence gel formation.\textsuperscript{17} At DA’s between 25% and 50%, the effects of electrostatic repulsion between GlcN\textsuperscript{+} monomers and hydrophobic association between GlcNAc monomers balance each other out.\textsuperscript{17} Evidence for distinct behaviors in these three regions comes from Montembault and coworkers who showed that increasing the DA from 5.2% to 52.1% decreases the time to form a gel from over 250 minutes to less than 100 minutes at a
chitosan concentration of 1.5% (w/w). They also observed that between 25% and 50% DA, the time to form a gel remains nearly unchanged. These results indicate that the hydrophobic association of the GlcNAc monomers must overcome the electrostatic repulsion of the GlcN$^+$ monomers in order for gels to form in solution. Researchers have also shown that the DA influences equilibrium properties of chitosan gels such as the storage modulus, which is related to the number of junctions between chains. Increasing DA leads to an increase in the storage modulus due to the hydrophobic interactions between GlcNAc monomers.

All-atom molecular simulations have been used to understand how chitosan’s functional groups affect molecular geometry and association in solution. Franca and coworkers performed atomistic molecular dynamics (MD) simulations of chitosan and chitin in aqueous solution using the GROMOS carbohydrate force field. Their results show that the dominant intra-molecular interaction in a chitin chain is the hydrogen bond between the hydroxide group on carbon three of the ring and the oxygen atom on the adjacent monomer (GlcNAc monomers). The dominant intermolecular interactions for chitosan are between the amine, acetyl, and hydroxymethyl groups (GlcN and GlcNAc monomers). Skrovstrup and coworkers performed atomistic MD and Monte-Carlo (MC) simulations using the AMBER force field to analyze the intra-molecular interactions between dimers, trimers, and tetramers consisting of combinations of GlcNAc and GlcN monomers. They studied how the flexibility of the molecules changes as a function of the monomer sequence, and observed that intra-molecular hydrogen bonding controls the stiffness of the molecule. Other groups have performed simulations of chitosan for applications in drug delivery, focused on the
ability of chitosan to encapsulate drug molecules, however their systems sizes were limited by the computational expense of all-atom simulations.

Coarse-grained models are often used to simulate complex systems, allowing investigation of larger systems over longer time scales than can be achieved in all-atom simulations. These models reduce the degrees of freedom of a molecule by grouping several atoms into a single coarse-grained site. To this point, little work has been done in developing coarse-grained models for chitosan. Kossovich and coworkers developed a hybrid coarse-grained/atomistic model of chitosan that groups some atoms into coarse-grained sites, but keeps others in their atomistic representation. This model uses a total of 7 spheres to represent each GlcN monomer, and is parameterized to accurately represent the interactions between chitosan and carbon nanostructures such as graphene nanoparticles and carbon nanotubes. The atomistic simulations used to derive the hybrid model were performed in AMBER, but Coulomb interactions were neglected because the carbon nanostructures are not charged. The authors found that the total molecular energies resulting from the atomistic and coarse-grained models differed by less than 1%, and the bulk densities of the chitosan molecules in the atomistic and coarse-grained models differed by less than 5%. Marrink and coworkers have developed a more general way to coarse-grain carbohydrates by extending the MARTINI force field. Monomers are broken into three coarse-grained sites, and the interaction potentials are assigned based on the four main types of interaction sites: polar (P), nonpolar (N), apolar (C), and charged (Q). Each main type is further broken down into sub-types based on their hydrogen bonding capability or polarity, such as their ability to act as a hydrogen bonding donor, acceptor, or both. This method of coarse
graining accurately reproduces many of the geometrical constraints on carbohydrate molecules including the torsional angles and radius of gyration in both water and nonane solvents. However, it does not include specific interaction potentials for chitosan or chitin. To this point we are unaware of any coarse-grained chitosan models that are appropriate for simulations of polymers containing a combination of GlcN and GlcNAc monomers.

The goal of our work is to develop a coarse-grained model of chitosan that can be used in simulations to predict how the DA affects its self-assembly in solution. The monomer sequences along the chitosan chains can be adjusted to pinpoint optimum chitosan compositions for various applications. A modeling tool that can quickly adjust the chitosan DA could prove to be useful for discovering the optimum conditions for synthesis of self assembled materials such as hydrogels for drug delivery.

In this paper, we describe the development of an implicit-solvent coarse-grained model for chitosan for use in large-scale simulations of chitosan in aqueous solution. This model includes representations for GlcN\(^+\) monomers and GlcNAc monomers. A chitosan chain of any molecular weight and monomer sequence can be built with a combination of these two monomers. Our simulations do not include a representation for GlcN because many experimental studies focus on conditions resulting in complete protonation of GlcN to GlcN\(^+\). Each monomer is modeled with 3 coarse-grained sites, and each site uses hard-sphere, square-well, and square-shoulder potentials to describe intermolecular and intra-molecular interactions. The parameters for each coarse-grained site are based on the results of atomistic simulations using the AMBER molecular dynamics package. Radial distribution functions (RDF) between both bonded and non-bonded coarse-grained sites are calculated.
from the atomistic simulation trajectory, and are used to calculate both the geometric and energetic parameters for use in the coarse-grained simulations. Interaction energies are developed using a modified iterative Boltzmann inversion of the atomistic RDFs between each coarse-grained site. Because our model excludes solvent, all interaction potentials are actually potentials of mean force. Chitosan stiffness is maintained through the use of pseudobonds, which control the bond fluctuations between coarse-grained sites based on the bond fluctuations observed in the atomistic simulations. Finally, the mass of each coarse-grained site is taken to be the total mass of all of the atoms contained in the coarse grained site. Discontinuous molecular dynamics (DMD) simulations, a fast alternative to traditional molecular dynamics (MD) which have been previously used to study a variety of biological and polymeric systems, are implemented to study the influence of chitosan DA on self-assembly in solution. To determine how chitosan composition affects network formation, we calculated the percolation probability over time for chitosan solutions with varying DA. Additionally we calculated the size of the largest network as a function of DA and performed an analysis of the average number of inter-chain monomer-monomer associations as a function of composition.

Highlights of our results are the following. Simulations started from a random initial configuration of chitosan chains show that increasing DA leads to a percolated network earlier in time than would otherwise occur. This behavior is consistent with experimental findings that increasing chitosan DA leads to a decrease in the time to form a gel. We also show that increasing DA leads to a decrease in the time it takes for all of the chains in the system to assemble into a single network. In systems containing varying concentrations of
chitosan at a set DA, we observe that the time to form a percolated structure decreases as the concentration is increased. Our results also indicate that the distribution of GlcNAc monomers along the chitosan chain can have a significant impact on the number of inter-chain monomer pairs formed during the simulation. Monomer sequences containing several GlcNAc monomers in a row show the formation of a greater number of interactions between GlcNAc monomers on different chains than those occurring for more evenly distributed sequences of GlcNAc monomers. This shows us that the sequence of GlcNAc monomers in addition to the DA may play a role in chitosan network formation in solution.

3.2 Methods

Our coarse-grained model consists of two monomer types: GlcN$^+$ and GlcNAc. Each of these monomer types contains 3 coarse-grained sites, leading to 6 total coarse-grained types. Figure 3.1 shows how the atoms are grouped into coarse-grained sites for each monomer type. Coarse-grained sites 1 and 4 contain carbons 1 and 4 on the ring and the oxygen that forms the glycosidic linkage between monomers. Sites 2 and 5 include the ring oxygen, the hydroxymethyl group, and carbon 5 on the ring. The main difference between these two monomer types is coarse-grained sites 3 and 6. Site 3 contains a protonated amine group while site 6 includes the acetyl group and therefore includes several more atoms than site 3. Our coarse-grained mapping is very similar to that used in the MARTINI carbohydrate force field. This coarse-graining scheme leads to a dramatic reduction in the number of sites on the chitosan chain, which can be seen in Figure 3.2. Each coarse-grained
site has a unique interaction potential with all of the other sites. The method for developing the interaction potentials will be discussed in detail later. This coarse-graining procedure could be applied to any polysaccharide by using a similar coarse-grained mapping. The methods for developing interaction potentials and geometric constraints that will be discussed later can be used for other polysaccharides, polymers, or even small molecules after the coarse-grained mapping has been established.

The coarse-grained model of chitosan is implemented in implicit-solvent discontinuous molecular dynamics (DMD) simulations. We used DMD because this method dramatically increases the speed of the simulations compared to traditional all-atom simulations. Unlike traditional molecular dynamics, which integrates Newton’s equations of motion at discrete time intervals, DMD is event-driven. The DMD algorithm searches for the soonest-to-occur event (collision between spheres), advances to the time that the collision occurs, and analytically calculates the new particle positions and velocities. An event occurs any time two particles reach a discontinuity in their interaction potential (hard-sphere, square-well, or square-shoulder) as a function of their separation distance. All particles in the simulation move linearly between collisions. The benefit of this event-driven technique is that an event occurs at every time step, rather than traditional MD simulations where many time steps can occur while molecules are beyond their interaction distance. The temperature of the system is controlled via the Andersen thermostat. The average system reduced temperature is defined to be $T^* = k_B T/\varepsilon_{\text{ref}}$, where $\varepsilon_{\text{ref}}$ is the reference interaction energy (which is taken to be $k_B T_{\text{ref}}$ where $T_{\text{ref}} = 297$ K), $k_B$ is the Boltzmann constant, and $T$ is the temperature. The Andersen thermostat regulates temperature using “ghost collisions”,
where a random particle in the system is chosen to collide with a ghost sphere to maintain the Maxwell-Boltzmann velocity distribution around the desired temperature. We performed all simulations using an in-house DMD simulation package.

DMD simulations of chitosan solutions are performed to investigate how the degree of acetylation of chitosan chains affects self-assembly. The sequence of GlcNAc monomers is random for all systems tested. The simulations begin at a high initial temperature of $T^* = 8.0$ for one billion collisions to randomize the system and eliminate any artifacts from the initial configuration. This high temperature equilibration is followed by a slow cooling to the set temperature of $T^* = 1.0$. The remainder of the simulation is at a constant temperature $T^* = 1.0$. The cooling and constant temperature portion of the simulation is run for a combined five billion collisions. The maximum network size, number of monomer-monomer associations, and percolation probability are calculated for the different systems to see how the chitosan composition influences self-assembly in solution. We define two coarse-grained sites to be interaction partners if they are on different chains and are within $1.5\sigma_{ij}$ of each other, where $\sigma_{ij}$ is the hard-sphere diameter between coarse-grained sites of type i and j. Two chains are considered to be in a network if any two coarse-grained sites on the two chains are interaction partners. The maximum network size is defined to be the number of chains in the largest network. The system is considered percolated if it contains a network that spans the entire length of the box in any direction, and the network connects back to itself across the periodic boundary condition. If a network in the system meets these criteria it is considered to be “infinitely large”. When the system is percolated at a specific time the percolation probability is equal to 1.0 at that time, if it is not percolated the percolation probability is
equal to 0.0 at that time. The final percolation probability is calculated by averaging the percolation data over time and a series of replicates.

The data used to calculate the parameters for our coarse-grained model was obtained from explicit-solvent NVT ensemble atomistic simulations using the AMBER molecular dynamics package. We performed two different types of atomistic simulations to develop the necessary parameters: (1) simulations of two separate monomers in water, and (2) simulations of a single, 6-mer chain in water. The total charge on any system containing protonated groups was neutralized by the addition of the appropriate number of acetic acid ions. We did not include any excess salt concentration in our simulations as is typically seen in experiments; therefore there was no screening of the charges. However, our coarse-grained model uses a relatively short cutoff distance between the charged sites (~2.5σij), and therefore implicitly accounts for screening of the electrostatic interactions. All simulations were run at 297K.

Explicit solvent atomistic simulations of pairs of monomers were performed to develop the intermolecular interaction potentials between the different monomer types. We chose simulations of monomers rather than polymer chains for this purpose to eliminate the effects of neighboring monomers in the chain. A total of 3 different simulations containing the following combination of monomers were used: (1) two GlcN+ monomers, (2) two GlcNAc monomers, and (3) one GlcN+ and one GlcNAc monomer. The oxygen atoms bonded to carbons 1 and 4, which form the glycosidic linkage between the monomers were not included because they are not easily accessible to chitosan monomers on other chains. The general AMBER force field (GAFF)38 was used for the monomer simulations and the
simulation box was 36Å x 36Å x 36Å. Each simulation was run for 190 nanoseconds; data for the radial distribution functions was collected over the final 180 nanoseconds.

The geometric constraints for the coarse-grained model were determined using explicit solvent atomistic simulations of isolated 6-monomer chains. The constraints are maintained by imposing a set of bonds and pseudobonds. The bonds used in our DMD simulations are different from those in traditional MD simulations because rather than having a potential energy that varies as a function of the bond distance, our method sets the minimum and maximum bond distance and allows the coarse-grained sites to fluctuate freely between these distances with no change in potential energy. Pseudobonds constrain the separation between non-bonded coarse-grained sites using the same methodology as for bonds. Five different simulations were required to develop all of the bond and pseudobond constraints for the different possible sequences. The different chitosan chain sequences used in the atomistic simulations are 1) X-X-X-X-X-X, 2) Y-Y-Y-Y-Y-Y, 3) X-Y-X-Y-X-X, 4) X-Y-Y-X-X-X, and 5) X-X-X-Y-Y-X, where X is a GlcN$^+$ monomer and Y is a GlcNAc monomer. This chain length was chosen to allow us to simulate all of the possible neighbor combinations for each monomer type for monomers in the middle of a chitosan chain. The box size for the chain simulations was 44Å x 44Å x 44Å. The GLYCAM_06h force field$^{39}$ was used for simulations of chitosan chains, and the simulations were run for 20 nanoseconds, with data being collected in the final 10 nanoseconds.
3.3 Results

Parameter Development

The interaction energies between coarse-grained sites were developed using a modified iterative Boltzmann inversion procedure. Recall that the standard iterative Boltzmann inversion procedure is a systematic method to develop coarse-grained interaction potentials based on RDFs from explicit solvent atomistic simulations. The first step in this method is to make an initial approximation for the coarse-grained interaction potential:

$$ U_{CG}^{(1)}(r) = -k_B T \ln g_A(r) $$

(1)

where $g_A(r)$ is the atomistic radial distribution function between the centers of mass of the groups of atoms making up the coarse-grained sites, $k_B$ is the Boltzmann constant, and $T$ is the temperature. Next, a coarse-grained simulation is run using the interaction potential, $U_{CG}^{(1)}(r)$, in Equation 1, and the RDF from this simulation, $g_{CG}^{(1)}(r)$, is compared to $g_A(r)$. If $g_{CG}^{(1)}(r)$ and $g_A(r)$ are within a certain acceptance criteria of each other, the potential is considered valid, if not the interaction potential is adjusted using:

$$ U_{CG}^{(i+1)}(r) = U_{CG}^{(i)}(r) - k_B T \ln \frac{g_{CG}^{(i)}(r)}{g_A(r)} $$

(2)

where $i$ is the iteration counter, $g_{CG}^{(i)}(r)$ is the coarse-grained RDF, $g_A(r)$ is the atomistic RDF, $k_B$ is the Boltzmann constant, and $T$ is the temperature. This procedure continues until $g_{CG}^{(i)}(r)$ converges to $g_A(r)$.

We have modified the procedure described above to make it appropriate for developing discontinuous potentials. The coarse-grained simulations used in the modified
iterative Boltzmann inversion procedure had the same box dimensions as those in the atomistic simulations and each contained two monomers. The solvent was implicit for all coarse-grained simulations and the reduced temperature was set to $T^* = 1.0$. Figure 3.3 shows sample discontinuous potentials and RDFs for each step of our modified iterative Boltzmann inversion procedure. Step 1: Calculate $g_A(r)$ from the atomistic simulation of a system containing the two coarse-grained sites under consideration. The $g_A(r)$ is shown in Figure 3.3a. Step 2: Choose the hard-sphere diameter and estimate the number of square-wells or shoulders required to reproduce $g_A(r)$ using a discontinuous potential. Hard-sphere diameters were set to be the first non-zero values of $g_A(r)$ followed by at least 10 non-zero values. The well boundaries were chosen based on the values of $g_A(r)$ at different values of $r$. The first well was meant to cover the region from the hard-sphere diameter to the lowest value of $r$ at which $g_A(r)$ was approximately 50% of its maximum value. The second well was meant to cover the region ranging from the end of the first well to the highest value of $r$ at which $g_A(r)$ was approximately 50% of its maximum value. The third well was meant to cover the region from the end of the second well to the cutoff distance. The above criteria were simply used as an approximation for the placement of square-well and square-shoulder boundaries; they were adjusted via visual inspection of the $g_A(r)$ between each coarse-grained site. The hard-sphere diameter is the first red, dashed line in Figure 3.3b, and the square-well/square-shoulder boundaries are the following three dashed lines. Step 3: Set the depths of all wells to zero (no attractions or repulsions). The red line in Figure 3.3c represents the hard-sphere interaction potential used for the first coarse-grained DMD simulation. Step 4: Run a DMD simulation using this interaction potential to calculate the
coarse-grained $g_{CG}^{(i)}(r)$, where $i$ is the iteration counter. The $g_{CG}^{(i)}(r)$ for $i = 1$ is shown in Figure 3.3d. Step 5: Compare the average value of $g_{CG}^{(i)}(r)$ within each square-well/square-shoulder region to the average value of $g_A(r)$ within the corresponding region (from the atomistic simulation). Figure 3.3e overlays $g_{CG}^{(i)}(r)$ (red) and $g_A(r)$ (black) for $i = 1$ and shows the square-well/square-shoulder boundaries. Step 6: Adjust the depth of each square-well/square-shoulder based on the percentage difference between the average values of $g_{CG}^{(i)}(r)$ and $g_A(r)$ within each square-well/square-shoulder region. (The wells are made deeper if $g_{CG}^{(i)}(r)$ is less than $g_A(r)$ and made shallower if $g_{CG}^{(i)}(r)$ is greater than $g_A(r)$. If the values of $g_{CG}^{(i)}(r)$ and $g_A(r)$ are less than 25% different, the well depth is adjusted by 0.025, if it is less than 75% different it is adjusted by 0.05, and if it is more than 75% different it is adjusted by 0.1.). Step 7: Repeat steps 4-6 until the average $g_{CG}^{(i)}(r)$ in each square-well/square-shoulder region matches $g_A(r)$ in the corresponding regions within 10%, while the number of wells/shoulders and widths of the wells/shoulders are held constant. Figure 3.3f shows the final $U_{CG}^{(i)}(r)$ on the left and $g_{CG}^{(i)}(r)$ (red) and $g_A(r)$ (black) on the right.

The final values of the hard-sphere diameters, $\sigma_{ij}$, square-well/square-shoulder boundaries, $\lambda_i$, and well depths, $\varepsilon_i$, between all possible coarse-grained sites are listed in Table 1. Note that not all interaction potentials contain the same number of wells. If the value for a well boundary and depth is marked with a dash it means there are no wells at that value of $\lambda$. If the only value reported is $\sigma_{ij}$, the interaction is a hard-sphere interaction.
Figure 3.4 compares the atomistic and coarse-grained RDFs between selected pairs of coarse-grained sites of type a) 4-4, b) 5-5, c) 5-6, and d) 6-6. It can be seen that the coarse-grained RDFs closely replicate those from atomistic simulations. We were able to accurately match atomistic RDFs between all coarse-grained sites using a maximum of four square-wells/shoulders per coarse-grained site.

The bond and pseudobond lengths are based on the RDFs between bonded and non-bonded coarse-grained sites from the atomistic simulations. The distance between two bonded coarse-grained sites that resulted in the maximum value of $g(r)$, $g(r)_{\text{max}}$, is denoted $r_{\text{max}}$. The minimum bond distance is taken to be the first value of $r$ (less than $r_{\text{max}}$) resulting in a $g(r)$ that is 50% of $g(r)_{\text{max}}$, while the maximum bond distance is taken to be the first value of $r$ (greater than $r_{\text{max}}$) resulting in a $g(r)$ that is 50% of $g(r)_{\text{max}}$. Three types of pseudobond constraints were applied to the chain: (a) backbone constraints, (b) angle constraints, and (c) torsional constraints. The backbone constraints and torsional constraints were developed in a similar way to the bond constraints, however the maximum and minimum pseudobond values were taken to be approximately the first values of $r$ greater than and less than $r_{\text{max}}$ resulting in a $g(r)$ that was 25% of $g(r)_{\text{max}}$ respectively. To maintain backbone stiffness we included next-nearest and next-next-nearest neighbor pseudobonds between the center beads of each monomer along the chain. The values of the average distances between neighbors, next-nearest neighbors, and next-next-nearest neighbors along the chain from both the coarse-grained and atomistic simulations can be seen in Table 2. The local stiffness of the chitosan backbone in the coarse-grained simulations is very close to that observed in atomistic simulations.
Pseudobond distances for the angle constraints were set based on the angle distributions observed from the atomistic simulation. Figure 3.5 compares the angle distributions calculated from our atomistic and coarse-grained simulations for a GlcN+ monomer between two GlcN+ monomers. The pseudobonds lead to a close match in the angle fluctuations seen in atomistic simulations despite allowing the coarse-grained sites to move freely between the minimum and maximum pseudobond distance. All bond and pseudobond parameters can be found in the supplemental information.

**Model Performance**

We began our analysis of the performance of the coarse-grained model by observing how the rate of chitosan self-assembly changes with DA. The simulations contained 77 chitosan chains (each 100 monomers long) in a cubic box with box lengths of 525 Å, yielding a chitosan concentration of 1.5 wt% and a packing fraction of 0.006. The wt% is defined as the total mass of chitosan divided by the total mass of the solution (chitosan + water) assuming the box is filled with water molecules. The packing fraction, \( \eta \), is defined as \( \eta = \pi N \sigma^3 / 6V \) where \( N \) is the number of coarse-grained sites, \( \sigma \) is the hard-sphere diameter of the coarse-grained site, and \( V \) is the volume of the box. We chose random placement of GlcNAc monomers along the chain to mimic experimental conditions where the monomer sequence is not typically controlled, and varied the DA from 10% to 50%. Figure 3.6 shows snapshots taken at the end of DMD simulations of chitosan systems at DA’s of 10%, 20%, 30%, 40%, and 50%. It is clear that increasing the DA leads to greater association of chains in solution. As the DA increases, the net positive charge on the chain decreases, leading to
less electrostatic repulsion between monomers. Additionally, the GlcNAc monomers increase the chain hydrophobicity and act as junction points for assembly. This phenomenon has been observed in experiments on chitosan where it has been shown that the time it takes to form a gel decreases with increasing DA.\textsuperscript{17}

The percolation probability, $\Pi$, is used as an indicator of chitosan gel formation in solution. Note that percolation does not guarantee that the system can be considered a gel, but is an indicator of “gel-like” behavior. To confirm chitosan gelation, rheological data on the viscous and elastic moduli is required. Figure 3.7 shows the percolation probability over time for systems with DA’s ranging from $10 - 50\%$ at a chitosan concentration of $1.5 \text{ wt}\%$. At a DA of $0\%$ the percolation probability is zero throughout the duration of the simulation, showing that GlcNAc monomers are necessary for association between chitosan chains. Since all of the monomers are protonated and therefore repulsive at a DA of $0\%$, the only significant association between chains would be in the form of entanglements. When the DA is increased to $10\%$, we observe an increase in the percolation probability, however it only reaches an average of approximately $0.25$. At this low DA there are not enough GlcNAc monomers to form a strong network, and therefore the system forms transient percolated structures that are not stable enough to remain intact throughout the duration of the simulation. A major transition in the percolation probability is observed once the DA reaches $20\%$ and beyond, where the percolation probability reaches a value of $1.0$. This is consistent with the behavior seen by Montembault and coworkers who observed a significant decrease in the time it takes to form a chitosan gel when the DA is increased from $6\%$ to $20\%$.\textsuperscript{17} At DA’s greater than $20\%$, the percolation probability increases earlier in time with each
increase in DA, demonstrating the importance of the GlcNAc monomers in self-assembly. To confirm that the percolation probability trends were not a result of finite size effects, we performed simulations of systems at the same DA’s and concentrations, but in a box with approximately double the volume. We observed the same trends in the percolation probability as a function of time and DA, indicating that our results were not due to finite size effects.

Another way to quantify chitosan network formation is by calculating the number of chains in the largest network in the system. Figure 3.8 shows the size of the largest network in the system (in terms of the number of chains in the network) over time for each DA. Note that the maximum possible size for the largest network is 77 chains because this is the total number of chains in the system. The variation in the size of the largest network over time is similar to that seen for the percolation probability. The maximum network size is approximately 5 chains for a 0% DA system, with these networks being formed due to polymer entanglements. When the DA increases to 10%, the maximum network size is approximately 20-25 chains, about one third of the chains in the system. A major transition is again observed when the DA increases to 20%. At this point the largest network in the system contains nearly all of the chains, indicating significant network formation. We also observe that the size of the largest network in the system increases earlier in time with each corresponding increase in DA.

Figure 3.8 also reveals the presence of a lag time before there is a sharp increase in the size of the largest network as a function of time. The lag time at 10% DA is significantly longer than at higher DA’s; in fact there is no sharp transition in the size of the largest
network over time. The lag time at 20% DA is significantly shorter than what is observed at 10% DA, reflecting the previously mentioned experimentally observed behavior of gel time as a function of DA. Systems with chitosan DA’s of 30% and 40% show a very similar behavior to each other as evidenced by their similar lag times and approach to equilibrium. The lag time at 50% DA is significantly shorter than for all other DA’s and shows a nearly linear increase in the maximum network size from the beginning of the simulations. This observation conceptually agrees with the experimentally observed behavior of chitosan as a function of DA where at DA’s less than 25% the chains act as a cationic polyelectrolyte, at DA’s of 25-50% there is a transition region where the effects of electrostatic repulsion between GlcN\(^{+}\) monomers and hydrophobic association between GlcNAc monomers balance out, and at DA’s of 50% and greater chains act like hydrophobic polymers with isolated charges.\(^{17}\)

We also varied the concentration of the chitosan solutions to determine how polymer concentration affects the percolation probability. Figure 3.9 shows the percolation probability over time for systems with a DA of 30% and a random sequence of GlcNAc monomers for chitosan concentrations ranging from 0.5 wt% to 2.0 wt%, corresponding to packing fractions of 0.002 to 0.008. Our results show that increasing polymer concentration leads to percolation earlier in time for each concentration tested. Additionally, our results show that the lag time associated with the increase in the percolation probability nearly disappears when the concentration is increased from 1.5 wt% to 2.0 wt%. We also observe that the percolation probability at 2.0 wt% starts at a higher initial value than all other concentrations, caused by chain entanglements at a high concentration. Montembault and
coworkers showed that increasing the chitosan concentration leads to a decrease in the time needed to form a gel. We show a similar behavior where the percolation probability increases at earlier times with increasing chitosan concentration. The disappearance of the lag time in the percolation probability when the concentration is increased from 1.5 wt% to 2.0 wt% is consistent with behavior observed by Montembault and coworkers where chitosan concentrations above 1.8 wt% lead to proportionally shorter gel times than those at lower concentrations.

In addition to analyzing the association of chitosan chains, we calculated the number of monomer-monomer associations in the system. Previous work has shown that the equilibrium storage modulus of chitosan gels increases with increasing DA, supposedly due to increased inter-chain association of GlcNAc monomers. Figure 3.10 shows the number of monomer-monomer associations as a function of DA averaged over the final one billion collisions of the simulation. Each column in Figure 3.10 is broken into three segments: 1) GlcN\(^+\)-GlcN\(^+\) associations (black), 2) GlcNAc-GlcNAc associations (red), and 3) GlcN\(^+\)-GlcNAc associations (blue). The total height of the column is the sum of all monomer-monomer associations. It is clear that increasing the DA results in a greater number of inter-chain monomer-monomer associations resulting in stronger network formation. The breakdown of monomer associations shows that GlcN\(^+\)-GlcN\(^+\) associations make up a lower percentage of the total monomer-monomer associations as DA increases. This is expected due to the electrostatic repulsion between these monomers. In contrast, the number of associations between two GlcNAc monomers contributes more to the total number of monomer associations with increasing DA due to the hydrophobic interactions between these
monomers. We observed frequent interaction between GlcNAc and GlcN\(^+\) monomers, regardless of DA. Two factors contribute to this result: 1) for all systems except 50% DA there are more GlcN\(^+\) monomers than GlcNAc monomers in the system, and 2) even at 50% DA there are twice as many possible combinations of monomer associations between a GlcN\(^+\) and GlcNAc than between two GlcNAc monomers.

To elucidate the impact of monomer sequence on the number of monomer associations, we compared the number of different types of monomer-monomer associations that form for different sequences of GlcNAc monomers at 50% DA. Figure 3.11a shows the five different monomer sequences considered, where the black segments represent GlcNAc monomers and the white segments represent GlcN\(^+\) monomers. Figures 11b, 11c, and 11d show the number of GlcN\(^+\)-GlcN\(^+\), GlcNAc-GlcNAc, and GlcN\(^+\)-GlcNAc associations, respectively for the five sequences. In Figure 3.11b, sequences 1 – 4 show a similar number of GlcN\(^+\)-GlcN\(^+\) associations, while sequence 5 shows a significant reduction in this type of interaction. Visual inspection of the monomer sequences in Figure 3.11a reveals that sequence 5 is the “blockiest” sequence of GlcNAc monomers, resulting in the greatest number of associations between GlcNAc monomers. Sequence 1 displays the fewest number of interactions between GlcNAc monomers due to its even distribution of GlcNAc and GlcN\(^+\) monomers. Figure 3.11d shows that the greatest number of interactions between GlcN\(^+\) and GlcNAc monomers occurs for sequence 2. We believe this occurs due to the alternating sequence of GlcNAc and GlcN\(^+\) monomers near the beginning and the end of the chain in sequence 2. Sequence 1 results in the fewest associations between GlcN\(^+\) and GlcNAc monomers, and the fewest total number of monomer associations, the latter likely because it
is the most uniform sequence of all the sequences tested. The even distribution of GlcN\(^+\) monomers and GlcNAc monomers leads to a balance between electrostatic repulsion and hydrophobic association.

Finally, we calculated the radius of gyration (Rg) and persistence length (Lp) of a single 800-mer chitosan chain at DA’s ranging from 0\% to 50\% to emulate dilute solution conditions. The Lp is equal to half the Kuhn length, \(b\):

\[
b = \frac{6(Rg)^2}{lDP}
\]

where \(l\) is the virtual bond length, and DP is the degree of polymerization.\(^{18,41}\) The virtual bond length, \(l\), was set to be 5.39 Å because that is the average bond length between two type 1 coarse-grained sites. Table 3 reports the Rg and Lp values for chitosan chains at different DA’s. The results show a decrease in both the Rg and Lp with increasing DA, which can be attributed to increased chain hydrophobicity. Our reported values of Rg are similar to those previously presented in the literature\(^{19,20}\), although our model slightly under-predicts the Rg at high DA (\(\geq 30\%\)). This may be due to the fact that experimental systems at high DA can form aggregates in solution, but our systems do not because we only consider a single chain. Our values of the Lp are also supported by previous experimental work, which reports chitosan Lp values ranging from 6 nm\(^{42}\) to 22 nm\(^{43}\) under similar ranges of DA.

### 3.4 Discussion and Conclusions

We have developed a new implicit-solvent coarse-grained model of chitosan for use in DMD simulations that allows for variation of the DA. The model consists of two different
monomer types: protonated glucosamine (GlcN\(^+\)) and N-acetylglucosamine (GlcNAc), each consisting of three coarse-grained sites. Square-well/square-shoulder interaction potentials were derived via a modified iterative Boltzmann inversion procedure based on the radial distribution functions between the different monomers from all-atom simulations. Geometric constraints were met through a combination of bonds and pseudobonds based on bond distance and angle distributions from the all-atom simulations of chitosan and chitin chains. All explicit solvent atomistic simulations were performed in the AMBER molecular dynamics package.

The results of discontinuous molecular dynamics simulations focused on determining how the chitosan degree of acetylation affects self-assembly in solution were presented. We first calculated the percolation probability of a chitosan solution as a function of the DA for a random sequence of GlcNAc monomers at an effective chitosan concentration of 1.5 wt%. Our results indicate that the coarse-grained model conceptually matches the experimentally observed rate of chitosan gel formation as a function of DA.\(^\text{17}\) As we increase DA we see a decrease in the time needed to form a percolated network. We also observe a transition from 0% to 20% DA where the percolation probability changes from approximately 0.0 to 1.0.

Another way that we quantify the extent of network formation as a function of DA is by calculating the number of chains in the largest network for each system. At low DA’s the system consists of several small, unconnected, networks, while at high DA’s the system forms a single network that includes all of the chitosan chains. Our model indicates that there are three ranges of DA which exhibit distinctly different self-assembly behavior: 1) 0 – 20% DA which shows a long lag time with very little increase in the max network size
during this lag phase, 2) 30 – 40% DA which shows a shorter lag time with a faster increase in the max network size during this lag phase, and 3) 50% DA which shows almost no lag phase with the max network size increasing in a linear fashion at short times. The behavior we observed in these different ranges of DA closely resembles the behavior seen by Montembault and coworkers over similar ranges of DA.\textsuperscript{17}

To determine the effect of polymer concentration on network formation, we performed simulations of 30% DA chitosan at concentrations ranging from 0.5 wt% to 2.0 wt%. We observed that increases in the polymer concentration led to the percolation probability increasing at earlier times. We also found a transition in the percolation probability when the concentration increases from 1.5 wt% to 2.0 wt%. At a concentration of 2.0 wt%, the percolation probability increases significantly earlier in time than at all lower concentrations. Our results are supported by work from Montembault and coworkers, who showed that the time needed to form a gel decreases more quickly at chitosan concentrations above 1.8 wt%.\textsuperscript{17}

In addition to quantifying network formation, we were interested in which monomer associations were most prevalent in the chitosan networks. First we calculated the number of associations between two GlcNAc monomers, two GlcN\textsuperscript{+} monomers, and one GlcNAc and one GlcN\textsuperscript{+} monomer to see which type of interaction was most common. For all DA’s we observed that the interaction between GlcNAc and GlcN\textsuperscript{+} monomers was most frequent, followed by associations between GlcNAc monomers, and then associations between GlcN\textsuperscript{+} monomers. The number of interactions between GlcNAc and GlcN\textsuperscript{+} monomers was highest because most of the systems had a greater number of GlcN\textsuperscript{+} monomers than GlcNAc.
monomers. The GlcNAc monomers on different chains associate with each other more frequently when there are blocky segments of GlcNAc monomers rather than when there is a more uniform distribution of GlcN\(^+\) and GlcNAc monomers.

In conclusion we have developed a coarse-grained chitosan model that can be used to predict self-assembly in solution as a function of DA. To the best of our knowledge this is the first coarse-grained model that has this capability. Our results conceptually agree with experimentally observed solution behavior of chitosan with respect to its DA. Our analysis of the monomer sequence at 50% DA has indicated that the monomer sequence can have a significant impact on self-assembly at high DA. We suspect that at lower DA’s the monomer sequence will have an even greater impact and we plan to investigate this in our future work. We are currently extending our model to include representations of neutral glucosamine (GlcN), which will give us a way to approximate pH effects on chitosan assembly. Additionally we plan to investigate the effect of hydrophobic modification chains on network formation, as this has been shown in previous experimental work to lead to interesting solution behavior.

Although our coarse-grained chitosan model has many advantages for predicting solution behavior, there are also several limitations. The first limitation is that we used an implicit-solvent approach, which does not accurately model the diffusion of molecules or hydrodynamics. Without water molecules present, the chitosan chains can move more quickly through space than they could in the presence of water. Another limitation of our model is that we do not have a way to correlate the simulation time to a real time because the molecules move in a straight line in a vacuum between collisions rather than through a
solvent. Additionally, the chitosan chain lengths we use are an order of magnitude shorter than what is commonly used in experiments, which allows the chains to move more quickly than they would experimentally. The model could be used to simulate longer chains, but these simulations would need to be run for excessively long times due to the slow dynamics of polymer systems. In general, we expect the results of simulating longer chains to be similar to those discussed in this work because the chains we studied in this paper were already significantly longer than the persistence length of chitosan. However, we would expect to see more entanglements between chains with increasing chitosan molecular weight, which means that percolation might occur for systems at lower DA. Despite these shortcomings, we feel that our model can be a useful tool in understanding how chitosan DA and monomer sequence can impact solution behavior.

3.5 Acknowledgements

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Figure 3.1. Coarse-graining scheme for different monomer types. Numbers refer to the coarse-grained site type.
Figure 3.2. Atomistic and coarse-grained representations of a chitosan chain consisting of 6 GlcN⁺ monomers
Figure 3.3. Steps in developing discontinuous interaction potentials using the modified iterative Boltzmann inversion procedure: a) RDF from atomistic simulations, b) atomistic RDF with well-boundaries marked with red dashed lines, c) hard-sphere potential for the initial step in the coarse-grained DMD simulations, d) RDF from the first iteration of the coarse-grained DMD simulations, e) overlay of coarse-grained (red) and atomistic RDF (black) after the first iteration, f) final discontinuous potential (left) and overlay of the final coarse-grained RDF (red) and atomistic RDF (black) (right).
Figure 3.4. Comparison of atomistic (black) and coarse-grained (red) radial distribution functions between coarse-grained sites of type (a) 4-4, (b) 4-5, (c) 5-6, and (d) 5-5
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Figure 3.6. Snapshots at the end of simulations of chitosan solutions with DA as shown. The sequence of GlcNAc monomers is random and the chitosan concentration is 1.5 wt%
Figure 3.7. Percolation probability of 1.5 wt% chitosan solutions with DA’s ranging from 0% to 50% and a random sequence of GlcNAc monomers
Figure 3.8. Maximum network size of 1.5 wt% chitosan solutions with DA’s ranging from 0% to 50% and a random sequence of GlcNAc monomers.
Figure 3.9. Percolation probability of chitosan solutions with 30% DA and a random sequence of GlcNAc monomers at concentrations ranging from 0.5 wt% to 2.0 wt%
Figure 3.10. Number of different inter-chain monomer pairs as a function of the chitosan DA
Figure 3.11. a) Monomer sequences used for the 50% DA chitosan systems, b) number of monomer pairs between two GlcN\(^+\) monomers for each monomer sequence, c) number of monomer pairs between two GlcNAc monomers for each monomer sequence, d) number of monomer pairs between a GlcN\(^+\) and GlcNAc monomer for each monomer sequence
Table 3.1. Hard-sphere diameter, well/shoulder boundaries, and well/shoulder depths between each coarse-grained type

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<th>$\lambda_1$ (Å)</th>
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Table 3.2. Average distance between monomers along the chain in all-atom (AA) and coarse-grained (CG) simulations. Distances reported for chitosan are between two type 1 sites, and distances reported for chitin are between two type 4 sites. Nearest neighbors refer to monomers that are next to each other, next-nearest neighbors refer to monomers separated by one monomer, and next-next-nearest neighbors refer to monomers separated by two monomers, along the chain.

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<th>Neighbor Type</th>
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<td>CG distance (Å)</td>
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<tr>
<td>Next-Next-Nearest</td>
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Table 3.3. Radius of gyration (Rg) and persistence length (Lp) of 800-mer chitosan chains with different DA

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<th>Rg (nm)</th>
<th>Lp (nm)</th>
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<td>47.93 ± 11.02</td>
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<tr>
<td>10%</td>
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<td>6.87 ± 0.02</td>
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<td>50%</td>
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CHAPTER 4

Effect of Monomer Sequence and Degree of Acetylation on Self-Assembly and Porosity of Chitosan Networks in Solution

Chapter 4 is essentially a manuscript by Steven W Benner and Carol K Hall accepted by Macromolecules.
Effect of Monomer Sequence and Degree of Acetylation on the Self-Assembly and Porosity of Chitosan Networks in Solution

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Abstract

Chitosan is a versatile biopolymer that can self-assemble in solution to form hydrogels and nanoparticles. It consists of two types of monomers: glucosamine (GlcN) and N-acetylglucosamine (GlcNAc). Chitosan self-assembly is controlled by a balance of interactions between these two types of monomers, GlcN which gets protonated in solution leading to electrostatic repulsion, and GlcNAc which contains an acetyl group, leading to hydrophobic and hydrogen bonding interactions. We present the results of discontinuous molecular dynamics (DMD) simulations aimed at understanding how the degree of acetylation (DA) and monomer sequence affect network formation in solution. Chitosans with DAs ranging from 10% to 50% and three different monomer sequences, random, evenly-spaced, and blocky, are studied. We show that chitosans with blocky sequences of GlcNAc monomers form percolated networks earlier in time than random and evenly-spaced sequences for all DAs tested. Analysis of the pore size distributions of the resulting chitosan networks shows that blocky sequences of GlcNAc monomers lead to larger pores than
random and evenly-spaced sequences for DAs less than or equal to 30%. The monomer sequence has little impact on pore size distribution when the DA is 40% or higher. Finally, we show that at low DA, chitosan networks allow free diffusion of small molecules through the network, but slow the diffusion of large molecules. At high DA, chitosan networks allow free diffusion of both large and small molecules. We conclude that controlling the monomer sequence of chitosan could be effective at controlling the structure of the resulting network.

4.1 Introduction

Chitosan is a versatile biopolymer that has shown promise as a hydrogel scaffold due to its biocompatibility, monomer sequence, and ability to be hydrophobically-modified. It is a polysaccharide derived from chitin, which consists of a random distribution of D-glucosamine (GlcN) and N-acetyl-D-glucosamine monomers (GlcNAc). The GlcN monomers are typically protonated in solution, resulting in positively charged GlcN+ monomers, while the GlcNAc monomers remain uncharged, and associate through a combination of hydrophobic and hydrogen bonding interactions. Chitosan’s amphiphilic functionality leads to interesting solution behavior that can be controlled by changing the fraction of GlcNAc along the chain, and hence the degree of acetylation (DA). Previous experimental studies have shown that increasing the DA leads to increased self-association of chains in solution, and can lead to the formation of gels. We previously developed a coarse-grained model for chitosan chains with representations for both GlcNAc and GlcN+ monomers, that shows increased self-assembly upon increasing the DA. That work focused
primarily on random sequences of acetylated monomers, and their ability to form self-assembled networks. Here, we use our coarse-grained model of chitosan to investigate how the sequence of GlcNAc monomers affects chitosan self-assembly in solution, the structure of the resulting hydrogel network, and the ability of molecules to diffuse within the network. To the best of our knowledge, the effect of monomer sequence on chitosan self-assembly has not been discussed previously in experimental or simulation studies. Thus we do not believe that there are any experimental methods of controlling the sequence of GlcNAc monomers. This work shows that the ability to do so could lead to a controllable solution behavior.

Hydrogels are a promising option for drug delivery systems that require control of the rate of drug release in the body. Hydrogels are three-dimensional networks formed via physical-association or chemical cross-linking of polymers in solution. Changing the polymer characteristics, such as the monomer sequence, net charge, or cross-linking agents, makes the networks customizable in terms of their pore size, solubility, and structural rigidity. Upon exposure to water, the polymers transition from a strongly associating state with close contact between chains, to a swollen state, which maximizes the number of favorable interactions between water and the hydrophilic polymers. For this reason, numerous studies have focused on loading hydrogels with drugs and monitoring how the drugs diffuse out of the network upon swelling. An important consideration in choosing candidates for hydrogel-based drug delivery is that the polymers used to prepare the hydrogels, and the chemistries used to trigger cross-linking, must be biocompatible and non-toxic.
Ionic cross-linking is a common method for generating hydrogels when polymers are charged. This is accomplished via the addition of small-molecule or polymer cross-linkers with a charge opposite to that of the polymer in solution. Lim and coworkers used β-[tris(hydroxymethyl) phosphino]propionic acid (THPP), an organophosphorous cross-linker, to form gels from elastin-like polypeptides under physiological conditions. This led to rapid gel formation (within 1 minute) and a gel whose strong mechanical properties are comparable to cartilaginous tissues. \(^{30}\) Draget and coworkers showed that a chitosan gel can be formed using a molybdate polyoxyanion cross-linker. The gels formed via this cross-linking method were highly-flexible and showed significant swelling in water with low ionic strength. \(^{31}\) Vimal and coworkers also took advantage of electrostatic cross-linking by using tripolyphosphate (TPP) to form chitosan gels for gene delivery applications in shrimp. \(^{32}\) TPP has been used in a number of other studies to promote the formation of hydrogels and nanoparticles due to its ability to strongly bind to cationic sites. \(^{33-36}\) Despite the effectiveness of ionic cross-linking in hydrogel formation, careful consideration must be given to the choice of cross-linking agent for in vivo applications due to concerns about possible toxicity. For this reason, hydrogel formation without cross-linking agents is a desirable alternative.

Physical association of amphiphilic polymers, which contain regions of hydrophobic and polar monomers, are commonly used to promote hydrogel formation without the need for cross-linking agents. The hydrophobic segments associate in aqueous solution to avoid unfavorable contacts with water molecules, leading to hydrophobic junctions that hold several polymer chains together in a network. The polar segments of the chain allow water and hydrophilic molecules to enter or exit the network. One example of a commonly used
amphiphilic polymer is Poloxamer 407 (an amphiphilic triblock copolymer in the polaxamer class of copolymers), which has been shown to effectively delay the release of Lidocaine in saline solution. This delayed release, caused by the time it takes for the drug to diffuse through the gel, leads to a longer duration of both motor and sensory nerve block in the sciatic nerve in rats. Other researchers have used non-poloxamer based (ABA) triblock copolymers to control the release of drugs, where A and B represent hydrophobic and hydrophilic blocks respectively. Triblocks consisting of poly(L-lactic acid) or poly(L-glycolic acid) with poly(ethylene glycol) (PEG) are commonly used in drug delivery because of their biocompatibility and because they show drug release over longer periods of time than polaxamer-based copolymers. Numerous other amphiphilic copolymers have also been used to form gels that enable controlled drug release.

Hydrogel formation via hydrophobic association of amphiphilic polymers is not limited to multi-block copolymers; comb copolymers can also be used to form hydrogels in solution. Dulong and coworkers showed that pullulan-based comb copolymers with hydrophobic grafts can be used to increase association between polymer chains and more effectively capture target molecules. They also showed that increasing grafting density of the comb copolymer led to a reduction in hydrogel swelling compared to a hydrogel without hydrophobic grafts. Chiu and coworkers found that adding hydrophobic grafts to chitosan leads to improved network formation over pure chitosan, leading to in-situ hydrogel formation. Their results also showed that increasing the density of hydrophobic grafts leads to increases in solution viscosity due to an increased number of hydrophobic junctions. Hydrophobically-modified chitosan (HMC) has also shown the ability to form gels in the
presence of hydrophobic nanoparticles\textsuperscript{48, 49} and vesicles\textsuperscript{48}, while chitosan without hydrophobic modification is unable to achieve this phenomenon.

The goal of this work is to understand how the DA and the sequence of GlcNAc monomers affects network formation in solution. We investigate: (1) how three different sequences of GlcNAc monomers: random, evenly-spaced, and blocky, can change the self-assembly of chitosan chains in solution and the structure of the resulting network, and (2) how the resulting pore size distribution affects the diffusion of molecules through the network. A modeling tool that can predict the structure of chitosan-based hydrogels and the diffusion of molecules within those hydrogels could be useful for applications in controlled drug delivery.

In this paper we present the results of implicit-solvent discontinuous molecular dynamics (DMD)\textsuperscript{50-52} simulations of chitosan hydrogel formation using our previously developed coarse-grained model for chitosan. The details of this model can be seen in our previous work.\textsuperscript{8} The intermolecular and intramolecular interactions between all coarse-grained sites are represented by a combination of hard-sphere, square-well, and square-shoulder potentials. Our previous work focused solely on the rate of network formation and the number of monomer-monomer interactions for random sequences of GlcNAc monomers with DAs ranging from 0-50%. This work focuses on understanding how the sequence of GlcNAc monomers impacts network formation in solution as measured by the percolation probability, the number of monomer-monomer associations, the pore size distribution, and the diffusion of molecules within the network for three types of monomer sequences. We also calculate the DA at which the solution behavior becomes independent of monomer
sequence. Comparing the aforementioned properties of the different networks provides insight into how the monomer sequence impacts both the rate of network formation and the structure of the networks.

Highlights of our results are the following. Simulations started from a random initial configuration of chitosan chains with varying DA and sequences of GlcNAc monomers show that as the blockiness of GlcNAc monomers increases, the network percolates earlier in time. At low DA, the only sequences resulting in percolated networks are the blocky sequences; random and evenly-spaced sequences do not form stable percolated structures. Blocky sequences of GlcNAc monomers lead to significantly more inter-chain monomer-monomer associations than random and evenly-spaced sequences, especially at low DA. At a DA of 40% or greater, the number of monomer pairs formed is nearly independent of the monomer sequence. Analysis of the pore size distribution of networks formed at various DA and monomer sequences reveals that at low DA, the chitosan chains are evenly distributed in solution due to electrostatic repulsion between chains, leading to small pores. At high DA, the chitosan chains associate more strongly due to increased hydrophobicity, resulting in large pores. Blocky sequences of GlcNAc monomers lead to larger average pore sizes than random and evenly-spaced sequences at 30% DA and lower, but not at higher DA. Finally, we see that chitosan networks at 10% DA allow the diffusion of small particles through the network, but slow the diffusion of large particles due to the small pore size distribution. Once the DA reaches 50%, there is free diffusion of both large and small particles due to the large pore size in the network.
4.2 Methods

The coarse-grained model includes representations for two monomer types: GlcN$^+$ and GlcNAc, both of which contain 3 coarse-grained sites. Figure 4.1 shows how the atoms are grouped into coarse-grained sites for each type of monomer. The method for developing the coarse-grained parameters for each monomer type is presented in our previous work. The mass of each coarse-grained site is taken to be the sum of the masses of the atoms in the site. Interaction potentials between coarse-grained sites are developed using an iterative Boltzmann inversion procedure modified to be applicable to discontinuous potentials. Iterative Boltzmann inversion is based on matching radial distribution functions between atomistic and coarse-grained simulations. Geometric constraints are derived from bond length and angle distributions between the different functional groups in the atomistic simulations. Nanoparticles are modeled as single spheres with diameters of 20 Å and 60 Å; they have hard-sphere interactions with all of the other coarse-grained sites. The mass of the nanoparticles is held constant at 500 amu regardless of the diameter to focus on how the size of the particle alone affects its diffusion within the networks.

The coarse-grained model was developed for use in implicit-solvent discontinuous molecular dynamics (DMD) simulations. DMD is an event driven technique, meaning the algorithm calculates the soonest to occur event, and advances to that time. An event occurs any time two coarse-grained sites reach a discontinuity in their square-well or square-shoulder potentials as a function of their separation distance. Therefore, important dynamic information is captured at every time step unlike traditional MD, which advances in time by
small, constant time steps. All particles in the system move linearly between collisions. The DMD method results in a dramatic increase in simulation speed over traditional molecular dynamics. The temperature of the system is maintained using the Andersen thermostat, which regulates temperature via “ghost collisions”. During a ghost collision a random particle in the system is chosen to collide with a ghost sphere to maintain the Maxwell-Boltzmann velocity distribution around the desired temperature. The average reduced temperature of the system is defined to be $T^* = k_B T / \varepsilon_{\text{ref}}$ where $\varepsilon_{\text{ref}} = k_B T_{\text{ref}}$, $T_{\text{ref}} = 297$ K, $k_B$ is the Boltzmann constant, and $T$ is the temperature. Constraints on the molecular geometry are met through a combination of bonds and pseudobonds that set the minimum and maximum distance allowed between two coarse-grained sites, which can otherwise move freely with no change in potential energy. This method of controlling the bond lengths and angles increases the speed of the simulation compared to a typical spring-like bond interaction potential, and was successfully implemented by Cheon and coworkers in the PRIME20 model.

DMD simulations of 100-mer chitosan chains are performed to determine how different chitosan DAs and sequences of GlcNAc monomers can affect network formation in solution. Three sequences of GlcNAc monomers: random, evenly-spaced, and blocky (blocks consist of five GlcNAc monomers in a row) are tested. Schematic representations of the three types of monomer sequences are given in Figure 4.2, where red monomers represent GlcNAc and blue monomers represent GlcN$^+$. All simulations begin at an initial reduced temperature of $T^* = 8.0$ for 1 billion collisions to randomize the system and remove any bias associated with the initial configuration. Thereafter, the system is cooled slowly to a reduced temperature of $T^* = 1.0$, followed by a constant temperature portion of the simulation at $T^* =$
1.0 during which data is collected. The cooling and constant temperature parts of the simulation are run for a combined 5 billion collisions. The concentration of the chitosan solutions is set to approximately 1.5 wt%, which is defined as the total mass of all of the chitosan chains divided by the total mass of the solution, assuming the simulation box is filled with water. The simulation box has dimensions of 525 Å x 525 Å x 525 Å and a packing fraction of \( \eta = \frac{\pi}{6V} \left( \sum n_i \sigma_i \right)^3 = 0.006 \) where \( i \) stands for the coarse-grained type, \( n_i \) is the number of spheres of type \( i \), \( \sigma_i \) is the hard-sphere diameter of a sphere of type \( i \), and \( V \) is the box volume.

We monitor chitosan network formation in a number of ways. Two chitosan chains are considered to be in the same network if there is at least one coarse-grained site on each chain within 1.5\( \sigma \) of the other, where \( \sigma \) is the hard-sphere diameter between the two sites. This information is used to calculate the percolation probability over time for each system as an indicator of gel formation. A network is defined to be percolated if it meets two criteria: 1) the network spans the entire length of the simulation box in any direction, and 2) the network connects back to itself across the periodic boundary.\(^\text{56, 57}\) A percolated network is considered to be “infinitely long”. Note that having a percolated network does not guarantee that the system is actually a gel; rheological data such as the elastic and viscous moduli are required to confirm gel formation. In addition to percolation we calculate the number of inter-chain monomer-monomer associations as a function of the DA and monomer sequence. We also calculate the pore size distribution by choosing random test locations in the simulation box and growing a sphere until it makes contact with one of the coarse-grained sites in the system. The diameter of the test sphere when it first contacts a chitosan sphere is
taken to be the diameter of the pore.\textsuperscript{58,59} A total of 500,000 random test points are chosen for each network to thoroughly cover the entire box. Finally we determine how the different pore sizes resulting from changes in DA affect the diffusion of a particle within the network. We calculate the mean squared displacement (MSD) of 20 Å and 60 Å diameter particles in 10\% and 50\% acetylated chitosan solutions with a blocky sequence of GlcNAc monomers. The MSD is given by:

\[
\text{MSD}(\Delta t) = \langle (r(t + \Delta t) - r(t))^2 \rangle
\]

where $\Delta t$ is the time interval, and $r(t)$ is the position of the particle at time $t$. The average $\langle \cdots \rangle$ designates an average over time and over several trajectories. Error bars on all data points represent the standard error of the mean, taken from three to five independent replicates at each state point.

4.3 Results

Chitosan network formation as a function of composition is quantified using the percolation probability, $\Pi$. Figure 4.3 shows the percolation probability of 1.5 wt\% chitosan solutions with DAs of 10\% to 30\% for blocky (blue), random (black), and evenly-spaced (red) sequences of GlcNAc monomers. Note that the blocky sequence of acetylation contain segments of five GlcNAc monomers in a row. As DA increases, there are more five monomer blocks of GlcNAc on the chain, not larger blocks. At 10\% DA it can be seen that random and evenly-spaced sequences of GlcNAc monomers do not lead to a stable percolated network, with percolation probabilities fluctuating around 0.25. Unlike the
random and evenly-spaced sequences of GlcNAc monomers, the blocky sequence leads to a stable, percolated network. This implies that having several GlcNAc monomers in a row is necessary to form a stable network. As the DA increases, the percolation probability depends less on the sequence of GlcNAc monomers. However, regardless of the DA, the blocky sequence of GlcNAc monomers leads to percolation earlier in time than the random and evenly-spaced sequences. It can also be seen that the data for a random sequence of GlcNAc monomers falls between the data for the blocky and evenly-spaced sequences. Random sequences resemble a hybrid of blocky and evenly-spaced sequences, with both blocky domains and evenly-spaced domains at different points along the chain. These results indicate that having segments containing several GlcNAc monomers in a row leads to a stronger association between chains and formation of a stable network earlier in time.

Varying the degree of acetylation and monomer sequence leads to networks that have different pore size distributions. The pore size distribution is important for applications in drug delivery, as the pores must be large enough to allow drugs to escape, but not so large as to allow rapid diffusion out of the network with no time delay. The effect of DA on pore size can be observed by comparing snapshots at the end of simulations for systems with low and high DA. Figure 4.4 shows snapshots at the end of simulations of systems with 10% (left) and 50% (right) DA chitosan with a blocky sequence of GlcNAc monomers. It can be seen that increasing the DA leads to significantly stronger association between chains, resulting in a coarser network with larger void spaces.

Figure 4.5 shows the pore size distribution of 1.5 wt% chitosan solutions with DAs ranging from 10% to 50% and a blocky sequence of GlcNAc monomers. It is clear from
Figure 4.5 that increasing the DA leads to a wider distribution in pore size. The black line in Figure 4.5 corresponds to the snapshot on the left in Figure 4.4 and the green line corresponds to the snapshot on the right in Figure 4.4. At low DA the chitosan behavior is dominated by electrostatic repulsion between GlcN\(^+\) monomers, and therefore the chains stay more evenly dispersed in solution, leading to smaller pores. Increasing the DA leads to increased hydrophobic association between monomers to reduce unfavorable contacts with water, resulting in larger void spaces in the network. We can see that the pore size distribution is centered around 4 nanometers at 10% DA and 6 nanometers at 50% DA. This information is important for deciding what chitosan monomer sequence would be optimal for capturing or delaying release of different types of molecules.

Next we look at how the sequence of GlcNAc monomers affects the pore size distribution at different DA. Figure 4.6 shows the pore size distribution for chitosan DAs ranging from 10% to 50% for random (black), evenly-spaced (red), and blocky (blue) sequences of GlcNAc monomers. At 10% DA we see that random and evenly-spaced sequences of GlcNAc monomers lead to nearly identical pore size distributions, while the blocky sequence leads to a broader distribution. As the DA is increased to 20%, each sequence of GlcNAc monomers has a unique pore size distribution, with evenly-spaced GlcNAc monomers leading to the smallest pores, followed by random and then blocky sequences. At 20% DA the pore size distribution for a random sequence more closely resembles that of an evenly-spaced sequence than a blocky sequence because there is still a low probability of forming blocks of GlcNAc monomers at such a low DA. At 30% DA the pore size distribution for a random sequence of GlcNAc monomers is between that of evenly-
spaced and blocky sequences, showing that there are likely some regions with blocky segments of GlcNAc monomers and some regions with more evenly-spaced GlcNAc monomers. When the DA is 40% or greater, the pore size distribution appears to be independent of the sequence of GlcNAc monomers. The effect of monomer sequence on pore size distribution decreases at high DA because there are so many hydrophobic monomers that there is a nearly uniform attraction along the chain regardless of monomer sequence. Overall, we can see that both the monomer sequence and the DA can have a significant impact on the resulting chitosan network that forms in solution.

The pore size distribution in the different chitosan networks affects the diffusion of molecules within the network. To observe this effect, we inserted a single sphere into a preformed chitosan network and calculated its mean squared displacement (MSD) over time. This indicates how far the particle travels over a set period of time. We performed this analysis on 10% and 50% acetylated systems with blocky sequences of GlcNAc monomers because these systems have significantly different pore size distributions. The test particle had only hard-sphere interactions with the chitosan chains, meaning changes in the MSD only occur when the particle collides with the network. Figure 4.7 shows the MSD over time for 20 Å (black) and 60 Å (red) diameter particles in (a) 10% DA, and (b) 50% DA systems with blocky acetylation. Note that the mass of the particle was held constant regardless of its diameter to remove mass effects from the MSD. It can be seen in Figure 4.7a that there is a noticeable decrease in the MSD of the particle when the diameter increases from 20 Å to 60 Å. This is to be expected because the most probable pore size in the 10% DA systems with blocky acetylation is 40 Å. Therefore particles with a diameter of 20 Å can diffuse freely in
the network while particles with a diameter of 60 Å collide more frequently with the chitosan chains. In contrast, Figure 4.7b shows that increasing the particle diameter from 20 Å to 60 Å in a 50% DA network with blocky acetylation does not lead to a noticeable change in the particle’s MSD. Because the pores are larger in the 50% DA network (the most probable pore size is 60 Å), the larger particle is able to diffuse similarly to the smaller particle. If we were to increase the particle diameter further we would likely see a decrease in the MSD.

Increasing the DA and the blockiness of the GlcNAc monomer sequence increases the number of inter-chain monomer-monomer associations. Figure 4.8 shows the total number of monomer associations for 1.5 wt% chitosan solutions with DAs of 10% to 50% and different sequences of GlcNAc monomers, averaged over the final one billion collisions of the simulation. The total height of each column represents the total number of monomer pairs formed. Each column is broken into the three parts, which represent different types of monomer associations: association between a GlcN⁺ and GlcNAc monomer (blue), association between two GlcNAc monomers (red), and association between two GlcN⁺ monomers (black). There are significantly more monomer associations for a blocky sequence of GlcNAc monomers than for random and evenly-spaced sequences at 10% DA and 20% DA. At 30% DA the number of monomer associations for a random sequence is closer to the blocky sequence than at lower DA, while the evenly spaced sequence shows far fewer associations. At 40% DA the total number of monomer-monomer associations is nearly identical for the three monomer sequences, however the distribution of the type of interactions is different. The blocky sequence leads to the greatest number of associations between GlcNAc monomers, followed by random, and then evenly-spaced. We see fewer
associations between GlcN\(^+\) monomers for blocky and random sequences than for evenly spaced sequences at high DA because there are larger gaps between regions of acetylation, allowing the GlcNAc monomers to associate with each other while the GlcN\(^+\) monomers stay further apart. At 50% DA the total number of monomer associations is the highest for the evenly-spaced sequence because the alternating sequence of GlcNAc monomers forces GlcN\(^+\) monomers into close proximity due to being surrounded by GlcNAc monomers. However, the blocky sequence still leads to the greatest number of associations between GlcNAc monomers and the fewest number of associations between GlcN\(^+\) monomers.

The GlcNAc monomer sequence affects the mechanism of chitosan self-assembly in solution. Figure 4.9 shows the average number of monomer-monomer associations for each monomer along the chain for (a) 10%, (b) 30%, and (c) 50% DA chitosan with blocky (left) and evenly-spaced (right) sequences of GlcNAc monomers. The black histograms show the monomer sequence, where black segments represent GlcNAc monomers and white segments represent GlcN\(^+\) monomers, while the red histograms show the average number of monomer-monomer associations for each monomer along the chain. Chitosans with blocky sequences of GlcNAc monomers display significant association between chains localized around the GlcNAc blocks as seen in Figure 4.9a-c (left). There is a significant reduction in the number of monomer associations in regions of GlcN\(^+\) monomers, confirming our explanation that blocky sequences allow GlcNAc monomers to associate while the GlcN\(^+\) monomers remain further apart. In Figure 4.9a-c (right), we see that evenly-spaced sequences of GlcNAc monomers lead to a more uniform association along the chain. We do not observe any spikes in the average number of monomer associations along the chain for an evenly-spaced
sequence of GlcNAc monomers, confirming that there is a uniform attraction along the chain. Evidently, the GlcN$^+$ monomers cannot stay separated from each other at high DA because the attraction between GlcNAc monomers overpowers the electrostatic repulsion, and forces the GlcN$^+$ monomers into close proximity.

4.4 Discussion and Conclusions

We have presented the results of discontinuous molecular dynamics simulations aimed at understanding how both the DA and sequence of GlcNAc monomers affect chitosan solution behavior. Chitosan with DAs from 10% to 50% and three different sequences of GlcNAc monomers: random, evenly-spaced, and blocky (5 monomer blocks) were studied. Throughout the course of the simulations we monitored the network formation of chitosan in a number of ways. First we calculated the percolation probability over time to determine how the DA and monomer sequence affected the ability of chitosan to form stable networks in solution. We observed that at 10% DA the blocky sequence of acetylation led to a stable percolated network, while the random and evenly-spaced sequences did not. At this low DA, random and evenly-spaced sequences did not have enough GlcNAc monomers adjacent to each other to form a long-lasting network, and resulted in a percolation probability that fluctuated around 0.25. When the DA was increased to 20% or higher, all sequences of acetylation led to a stable percolated network, but the blocky sequence of GlcNAc monomers percolated the earliest in time, followed by the random and evenly-spaced sequences. Our results regarding increasing percolation probability with increasing DA are supported by
several experimental studies, which show an increased tendency to form a chitosan gel with increasing DA. \textsuperscript{1,5}

Next we looked at the number of inter-chain monomer-monomer associations that occurred as a function of DA and monomer sequence. At 10\% and 20\% DA we saw that blocky sequences of GlcNAc monomers led to significantly more monomer associations than random and evenly-spaced sequences. This showed that at low DA it is important for several GlcNAc monomers to be in a row to promote association between chains. At 30\% DA the blocky sequence of acetylation still led to the greatest number of monomer associations, but the random sequence was a close second. As the DA increased, more densely acetylated domains began to occur along the chain, making its behavior more similar to a blocky sequence than an evenly-spaced sequence. At 40\% DA a transition occurred where the total number of monomer-monomer associations was nearly the same regardless of the monomer sequence. Interestingly, the evenly-spaced sequence of GlcNAc monomers led to the most monomer-monomer associations at 50\% DA, while the blocky sequences showed the fewest. This occurred because the blocky sequence had regions of GlcNAc monomers separated by regions of GlcN\textsuperscript{+} monomers, allowing the GlcNAc monomers to associate while the GlcN\textsuperscript{+} monomers stayed further apart. The evenly-spaced sequence had an alternating sequence of GlcNAc and GlcN\textsuperscript{+} monomers at 50\% DA, forcing the GlcN\textsuperscript{+} monomers to associate.

We also calculated the pore size distributions for different chitosan networks as a function of DA and monomer sequence. We showed that for a blocky sequence of GlcNAc monomers, the pore size increased with increasing DA due to the greater hydrophobicity of the chain. At 10\% to 30\% DA we saw that the blocky sequence led to the largest pore size
distribution, followed by the random and then evenly-spaced sequences. At 40% DA or greater, the pore size distributions were very similar regardless of the monomer sequence. This behavior aligned with the data showing that the number of monomer-monomer associations at 40% and 50% DA was very similar for all of the monomer sequences.

Finally we looked at how the chitosan networks impacted the diffusion of different sized particles within the network. We compared the mean squared displacement of 20 Å and 60 Å diameter particles in 10% and 50% DA networks with a blocky sequence of GlcNAc monomers. The 10% DA network resulted in a decrease in the particle’s mean squared displacement with an increase in the particle diameter, while the 50% DA network showed a nearly identical mean squared displacement for both particle sizes. The pore size distribution in the 10% DA network was centered around 4 nm (40 Å) in diameter, meaning that the 20 Å particle could freely move through these pores, while the 60 Å particle collided with the network more frequently. The pore size distribution at 50% DA system was centered around 6 nm (60 Å), which allowed for relatively free movement of both 20 Å and 60 Å particles through the pores.

In conclusion we have found that the chitosan monomer sequence can have a significant impact on its solution behavior at different DAs. These results could be used to design chitosan hydrogels for applications in drug delivery by adjusting the monomer sequence to control pore size distributions and mechanical properties. Adjusting the pore size distribution could be valuable in situations where it may be desirable to administer two drugs simultaneously and control whether they release simultaneously or at different times. The intermolecular interactions between the polymer network and the drug molecules would
also have significant impact on diffusion, but that is beyond the scope of the current work. We plan to investigate this phenomenon in more detail in the future. We also plan to extend this model to include representations for hydrophobic modification chains consisting of alkanes to learn how modification chain length and modification density can be used to control network formation.

Although our coarse-grained simulations provide insight into the effect of composition on solution behavior, the methods used have some limitations. The first limitation is that the model uses implicit solvent, which does not provide an accurate representation of molecular diffusion or hydrodynamics. Without the presence of water molecules, the solute can travel more quickly through space than it can with water molecules present. The second limitation is that it is difficult to make a direct correlation between the reduced temperature and the real temperature. The coarse-grained parameters were developed from atomistic simulations at room temperature, so we are unsure of how accurately the model would predict behavior at physiological temperature. The third limitation is that it is difficult to correlate the reduced time in the simulation to the real time because the molecules move in a straight line in a vacuum between collisions, rather than traveling in a solvent. To make a comparison of reduced time to real time we would need to compare behavior in our model to experimental behavior for a more realistic correlation.
4.5 Acknowledgements

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4.6 References


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Figure 4.1. Coarse-grained representations for both monomer types. Numbers refer to the coarse-grained site type.
**Figure 4.2.** Schematic representations of random, evenly-spaced, and blocky sequences of GlcNAc monomers. Red monomers represent GlcNAc monomers and blue monomers represent GlcN⁺ monomers.
Figure 4.3. Percolation probability for 1.5 wt% chitosan solutions with (a) 10%, (b) 20%, and (c) 30% DA and blocky (blue), random (black), or evenly-spaced (red) sequences of GlcNAc monomers.
Figure 4.4 Snapshots at the end of simulations of 10% (left) and 50% (right) DA chitosan systems at a concentration of 1.5 wt% with a blocky sequence of GlcNAc monomers. The colors of each coarse-grained site correspond to the colors shown in Figure 4.1.
Figure 4.5. Pore size distributions of 1.5 wt% chitosan solutions with DAs ranging from 10% to 50% and a blocky sequence of GlcNAc monomers
Figure 4.6. Pore size distributions for 1.5 wt% chitosan solutions with DAs ranging from 10% to 50% and random (black), evenly-spaced (red), and blocky (blue) sequences of GlcNAc monomers
Figure 4.7. Mean squared displacement of particles with diameters of 20 Å (black) and 60 Å (red) in 1.5 wt% chitosan solutions with DAs of (a) 10% and (b) 50% for a blocky sequence of GlcNAc monomers. Shaded areas represent the error associated with the data.
Figure 4.8. Number of monomer-monomer associations between a protonated and acetylated monomer (blue), two GlcNAc monomers (red), and two GlcN\textsuperscript{+} monomers (black) for systems with (a) 10\%, (b) 20\%, (c) 30\%, (d) 40\%, and (e) 50\% DA and random, even, and blocky sequences of GlcNAc monomers
Figure 4.9. Average number of monomer associations for each monomer on (a) 10%, (b) 30%, and (c) 50% acetylated chitosan chains with blocky (left) and evenly-spaced (right) sequences of GlcNAc monomers. Black histograms show the monomer sequence, where black segments represent GlcNAc monomers and white segments represent GlcN+ monomers. Red histograms show the average number of monomer-monomer associations for each monomer on the chain.
CHAPTER 5

Nanoparticle Induced Assembly of Hydrophobically-Modified Chitosan

Chapter 5 is essentially a manuscript by Steven W Benner and Carol K Hall submitted to Soft Matter.
Nanoparticle Induced Assembly of Hydrophobically-Modified Chitosan

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Abstract

Hydrophobically-modified chitosan (HMC) self-assembles in solution to form gels, making it suitable for applications in oil dispersion, hydrogel design, and wound dressing. The self-assembly of HMC is driven by the association of hydrophobic moieties that are attached to chitosan monomers along the polymer chain. We present the results of discontinuous molecular dynamics (DMD) simulations aimed at understanding how the length and density of the hydrophobic modification chains attached to HMC affect self-assembly and the structure of the resulting network. Long modification chains are required to promote the formation of a stable network in solution at a modification density of 5%; the networks form more readily at a modification density of 10%. The pore size distribution of the resulting HMC network is relatively independent of the modification chain length and density. Insertion of different sized hydrophobic nanoparticles into HMC has a significant impact on network formation, with the particles acting as junction points that promote the association of several HMC chains. The networks form faster in the presence of many small
nanoparticles than in the presence of few large nanoparticles. We conclude that HMC could be a viable candidate to form hydrogels in solution, and that the HMC architecture can be adjusted to control the strength of the network and rate of network formation both in the presence and the absence of hydrophobic particles.

5.1 Introduction

Hydrophobically-modified chitosan (HMC) has shown promise in biomedical and environmental applications due to its ability to self-assemble in solution. Chitosan is a naturally occurring polysaccharide consisting of a random sequence of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) monomers. GlcN monomers contain a primary amine functional group, which is typically protonated in solution to form positively charged GlcN\(^+\) monomers. The net positive charge of chitosan chains in solution increases their solubility, but causes the chains to repel each other. Hydrophobic moieties can be added to the GlcN monomers via a reductive amination reaction on the primary amine group, making the polymer amphiphilic and promoting self-assembly in solution. Hydrophobic modification can be adjusted by changing the modification chain length (number of atoms in the hydrophobic group) and the modification density (the percentage of GlcN monomers on the chitosan chain that contain hydrophobic groups). Molecular simulations can be used to learn how different lengths and quantities of hydrophobic groups affect assembly in solution. In this paper we expand our coarse-grained model of chitosan to include hydrophobic modification chains, and show how the modification density and modification chain length
affect the ability of HMC to form percolated networks in the presence of hydrophobic particles.

The ability of HMC to assemble in solution makes it a viable candidate for hydrogel drug delivery applications. Hydrogels are three-dimensional polymer networks formed in solution through physical or covalent interactions. Amphiphilic polymers are often used to form hydrogels in aqueous solution because the hydrophobic regions associate while the hydrophilic regions remain in solution. Chiu and coworkers showed that adding hydrophobic palmitoyl groups to chitosan led to self association via the formation of hydrophobic junction points between the hydrophobic groups. They also showed that increasing the number of hydrophobic moieties on the chitosan chain resulted in increasing solution viscosity due to an increased number of physical crosslinks between chains. Kjøniksen and coworkers also reported an increase in solution viscosity with increasing modification density using dodecane modification chains, again correlating this behavior to increasing crosslinks between hydrophobic side chains. HMCs have also been used to form nanoparticles that act as drug carriers. Zhang and coworkers hydrophobically modified chitosan with oleoly chloride to encapsulate the cancer drug doxorubicin. They showed that HMC was able to capture 52.6% of doxorubicin, and was effective at releasing the drug when the solution pH was lowered.

HMC assembly in solution can be enhanced in the presence of other nano- and microstructures. Dennis and coworkers showed that the addition of HMC to a solution of carbon microspheres leads to rapid gelation, which does not occur in the microspheres’ absence. They also noted that a solution of unmodified chitosan with carbon microspheres
does not lead to gel formation. Chen and coworkers showed that a water-soluble form of HMC led to the gelation of both anionic vesicles and PMMA nanoparticles. A solution of unmodified chitosan in the presence of the same vesicles and nanoparticles did not lead to gelation. Similar behavior was seen by Dowling and coworkers who studied HMC as a blood-clotting agent. The modification chains of HMC anchored into the red blood cell membrane, leading to the formation of a gel, which acts as a blood clot. Blood did not clot in the presence of unmodified chitosan. Sprayable foams of HMC can also be used to stop hemorrhaging in wounds that are not compressible.

HMCs have also been studied for potential applications in oil spill remediation. Venkataraman and coworkers showed that HMC could be an effective oil dispersant additive, which can either prevent oil aggregation, or promote oil gelation depending on the molecular weight of chitosan. At low molecular weight (50 – 190K Da), several HMC chains wrapped around an oil droplet anchored by the hydrophobic modification chains, and prevented its coalescence due to the electrostatic repulsion between chitosan backbones. At high molecular weight (310 – 375K Da), HMC chains anchored into several oil droplets at a time and led to the formation of a chitosan/oil gel. Simulations of HMC and oil showed that the anchoring of hydrophobic modification chains into oil droplets led to a larger oil surface area over time, and therefore acted effectively as an oil dispersant additive. Chitosan without hydrophobic modification chains had minimal effect on preventing oil aggregation.

The goal of this work is to use our coarse-grained model of chitosan to study how hydrophobic modification affects solution behavior in the presence and absence of nanoparticles. We vary the modification chain length and the modification density to
understand how HMC self-assembly changes as a function of these parameters. We also investigate how the modification chain length and diameter of hydrophobic nanoparticles impacts the formation of HMC/nanoparticle networks. Understanding how hydrophobic modification affects self-assembly is important for designing HMCs for various applications. Our coarse-grained model allows us to quickly investigate a wide range of parameters to gain insight into optimum design of HMCs for specific applications.

In this paper we present the results of implicit-solvent discontinuous molecular dynamics (DMD) simulations of HMC self-assembly in solution using our coarse-grained model of chitosan. The details of this model can be found in our previous work. We have extended our chitosan model to include representations for hydrophobic modification chains based on alkanes. The intermolecular and intramolecular interactions between coarse-grained sites are described by a combination of hard-sphere, square-well, and square-shoulder potentials. Our simulations focus on HMCs in solution both in the presence and absence of hydrophobic nanoparticles. We look at how varying the modification density and modification chain length of HMC in aqueous solution affects its self-assembly in terms of the percolation probability, number of interactions between modification chains, and pore size distribution of the resulting network. We also investigate how the addition of hydrophobic nanoparticles of various sizes affects the self-assembly of HMC in solution. Finally, we calculate the percolation probability of solutions of HMCs and nanoparticles, and the number of contacts between modification chains and nanoparticles as a function of modification chain length and nanoparticle diameter.
Highlights of our results are the following. Simulations starting from a random initial configuration of HMCs in solution show that increasing the modification chain length and modification density lead to increased self-assembly in solution due to association of the hydrophobic modification chains. The pore size distribution of HMC networks remains independent of the modification chain length at low modification densities. As the modification density and chain length increase, the pore size increases due to increased hydrophobic association between chains. The addition of hydrophobic nanoparticles to the HMC solutions enhances self-assembly. The hydrophobic particles act as junctions for assembly of the modification chains and allow the HMCs to more easily form large, percolated networks. The size of the hydrophobic nanoparticle has a significant impact on the self-assembly behavior of the solution. Systems containing many small nanoparticles form networks more easily and show increased association between the nanoparticles and modification chains compared to systems containing few large nanoparticles. We also observe a “saturation” modification chain length above which there is no further increase in the self-assembly of HMC/nanoparticle solutions.

5.2 Methods

The coarse-grained grained model includes representations for three chitosan monomer types: protonated glucosamine (GlcN\(^+\)), N-acetylglucosamine (GlcNAc), and neutral glucosamine (GlcN), with each monomer consisting of three coarse-grained sites. The coarse-graining of GlcN\(^+\) and GlcNAc monomers is the same as that presented in our
previous work. The GlcN monomer uses the same grouping of atoms as the GlcN\textsuperscript{+}, however there is one less hydrogen on the nitrogen atom. The hydrophobic modification chains are based on alkanes, where each coarse-grained site represents three carbon atoms and their corresponding hydrogen atoms. Figure 5.1 shows how the atoms are grouped into coarse-grained sites for each type of monomer (top), and the final coarse-grained HMC representation (bottom). Note that although the bottom image is to scale, it only represents a portion of the chain, not the entire chain length. The mass of all of the coarse-grained sites is the cumulative mass of all of the atoms on that site. Interaction potentials between coarse-grained sites were developed using the iterative Boltzmann inversion procedure modified for discontinuous potentials that was described in our previous work. The interaction potentials between GlcN and GlcN\textsuperscript{+} or GlcNAc monomers were derived using the same coarse-graining approach as we used previously. Atomistic simulations were performed on two individual monomers in a box filled with water to calculate monomer-monomer radial distribution functions. Then DMD simulations were performed on the coarse-grained representations of these monomers, and the interaction potentials between the coarse-grained monomers were adjusted until the coarse-grained radial distribution function matched the atomistic radial distribution function. The interactions between the alkanes and the chitosan chains were derived from atomistic simulations of a single dodecane chain and a single 4-mer chitosan chain in a box filled with water again by matching radial distribution functions. Geometric constraints were met through a combination of bonds and pseudobonds based on bond and angle distributions calculated from atomistic simulations of chitosan and alkanes in
water. All of the hard sphere diameters, interactions energies, and geometric constraints between coarse-grained sites are listed in the supplemental information.

Nanoparticles are represented as single spheres with diameters of 40 Å, 60 Å, and 80 Å. The nanoparticles have hard-sphere interactions with each other and with all other spheres in the system except for the hydrophobic modification chains, with which they have a square-well attraction consisting of two steps. The strength of the attraction between the nanoparticles and modification chains was adjusted so that the HMC/nanoparticle network percolated at similar conditions as observed in previous experimental work on HMC/nanoparticle networks with 12-carbon modification chains. The masses of the nanoparticles are based on the density of PMMA, which is 1.18 g/cm³.

The coarse-grained model of chitosan was developed for use with implicit-solvent DMD simulations. DMD is a fast alternative to traditional molecular dynamics (MD) that allows the simulation of larger systems over longer time scales. Unlike traditional MD, which uses a continuous interaction potential such as a Lennard-Jones potential, DMD uses a discrete potential that consists of hard-sphere, square-well, and square-shoulder interactions. The simplified interaction potentials allow analytical calculation of collision dynamics unlike the numerical methods used in traditional MD. A discrete potential also allows the method to be event-driven, meaning the time until the next collision event (time when two coarse-grained sites reach a discontinuity in the interaction potential as a function of their separation distance) is calculated, and the system is advanced to that time. Therefore, important dynamic information occurs at every time step, unlike traditional MD, which advances in time using a small, constant time step. All particles in the system move linearly between
collisions in the DMD method. The temperature of the system is maintained with the Andersen thermostat, which regulates the temperature of the system via “ghost collisions”. When a ghost collision occurs, a random particle in the system collides with a “ghost” particle to maintain the Maxwell-Boltzmann velocity distribution around the desired temperature. The average reduced temperature of the system is expressed as \( T^* = \frac{k_B T}{\varepsilon_{ref}} \), where \( \varepsilon_{ref} = k_B T_{ref} \), \( T_{ref} = 297 \) K, \( k_B \) is the Boltzmann constant, and \( T \) is the temperature.

Geometric constraints were met through a combination of bonds and pseudobonds, which set the minimum and maximum distance allowed between two coarse-grained sites; these coarse-grained sites can otherwise move freely with no change in potential energy.

DMD simulations were performed to determine how varying the length and quantity of hydrophobic modification chains affects the self-assembly of HMCs in solution. Modification densities of 5% and 10%, and modification chain lengths of 4, 6, and 8 spheres were simulated. The modification density is defined as the percentage of monomers along the chain that contain a hydrophobic modification chain. All simulations begin at an initial high reduced temperature of \( T^* = 8.0 \) for 1 billion collisions to remove any artifacts from the initial configuration. The system is then slowly cooled to a reduced temperature of \( T^* = 1.0 \). The simulations are then run at a constant temperature of \( T^* = 1.0 \) and data is collected during this constant temperature segment of the simulation. The cooling and constant temperature parts of the simulation are run for a combined 5 billion collisions.

We determine the effect of varying the modification chain length and modification density on self-assembly in several ways. First, we define two HMC chains to be in a network if there is at least one coarse-grained site on one chain that is within \( 1.5\sigma \) of one
coarse-grained site on a different chain, where $\sigma$ is the hard-sphere diameter for that pair of coarse-grained sites. The system is percolated if there is at least one network in the system that spans the entire simulation box in any direction, and connects back to itself across the periodic boundary condition. If the system is percolated, the percolation probability at that time is 1, if not, it is 0. The percolation probability is averaged over time to observe the transition from a random system of HMC chains to a percolated network. Percolation is an indicator of “gel-like” behavior, but does not guarantee that the system is a gel (rheological data such as the elastic and viscous moduli are required to confirm gelation). Next we calculate the number of interactions between modification chains on different HMCs for each modification chain length and modification density to observe their tendency to self-assemble. We then calculate the pore size distribution for networks with various modification chain lengths and modification densities. We also calculate the percolation probability for systems containing different size nanoparticles to understand how nanoparticles affect HMC self-assembly. Finally, the number of associations between the hydrophobic modification chains and the nanoparticles is calculated to determine how the length and number of modification chains affect the ability of HMC to form networks in the presence of nanoparticles.

5.3 Results

First we look at systems of HMC without hydrophobic nanoparticles to understand how modification chain length and modification density affect network formation. Figure
5.2 shows the percolation probability, Π, over time for 1.5 wt% systems that are (a) 5% modified and (b) 10% modified with 4, 6, and 8 sphere modification chains, where wt% = mass of HMC / total mass of solution (HMC + water) assuming the box is filled with water molecules. This concentration corresponds to a packing fraction of $\eta = \frac{\pi}{6V} \left( \sum n_i \sigma_i \right)^3 = 0.007$ where $i$ stands for the coarse-grained type, $n_i$ is the number of spheres of type $i$, $\sigma_i$ is the hard-sphere diameter of a sphere of type $i$, and $V$ is the box volume. Figure 5.2a shows that long modification chains are necessary to promote network formation at a modification density of 5%. HMCs with 4- and 6-sphere modification chains do not form stable percolated networks but HMCs with 8-sphere modification chains lead to a stable, percolated network that remains intact throughout the simulation. The short modification chains do not lead to a stable percolated network because all of the monomers that are not hydrophobically modified are positively charged. The hydrophobic attractions between the modification chains are insufficient to overcome the electrostatic repulsion of the GlcN$^+$ monomers.

Figure 5.2b shows that HMC’s with a modification density of 10% can form stable percolated networks with modification chain lengths of 6 and 8 spheres. Modification chain lengths of 4 spheres lead to a percolation probability that fluctuates around 0.2, similar to that seen with 6-sphere modification chains with 5% modified HMC. This shows that a stable percolated network is not able to form at this modification chain length. It can also be seen that 10% modified HMC’s with 6- and 8-sphere modification chains show nearly identical percolation behavior with time. Both systems show an initial delay in the increase of the percolation probability, followed by a rapid approach to a percolation probability of 1.0. This rapid increase shows that the hydrophobic attraction between the modification chains
overpowers the electrostatic repulsion between the GlcN\(^+\) monomers once the modification chains are proximate to each other.

Next we investigate how the modification chain length and modification density affect the number of pairwise interactions between modification chains. A pair of modification chains are defined to interact if there is at least one coarse-grained site on the first modification chain that is within 1.5\(\sigma\) of a coarse-grained site of a modification chain on a different HMC molecule. If more than one set of coarse grained sites on a pair of interacting modification chains interact we would still count this as one pair of interacting chains. The interaction between modification chains is the only significant driving force for association between chains because all other monomers are positively charged. Figure 5.3 shows the average number of pairwise interactions between modification chains for 5\% and 10\% modified HMC during the final billion collisions of the simulation. It is clear from Figure 5.3 that HMCs with 4-sphere modification chains do not experience a significant number of pairwise interactions for both 5\% and 10\% modified HMC. A combination of the stiffness of the chitosan chain and the repulsion between the GlcN\(^+\) monomers makes it difficult for these modification chains to interact with each other. Increasing the modification chain length to 6 spheres leads to a notable increase in the number of pairwise interactions between modification chains for both modification densities. HMC solutions with 8-sphere modification chains experience nearly 3.5 times more pairwise interactions than 6-sphere modification chains. It is also interesting to note that despite the fact that there are many more pairwise interactions between modification chains for 10\% modified HMC than 5\% modified HMC (due to there being twice as many modification chains), the
increases in the number of pairwise interactions with respect to the modification chain length are proportional to each other. For example, the number of pairwise interactions between modification chains increases approximately 3-fold when the modification chain length is increased from 4 to 6 spheres for 5% modified HMCs. We see a very similar 3-fold increase for 10% modified HMC when increasing from 4- to 6-sphere modification chains.

The pore size distribution was calculated as a function of the modification chain length and modification density to learn more about the structure of the HMC networks. Figure 5.4 shows the pore size distributions for networks of HMC’s that are (a) 5% and (b) 10% modified with 4- (black), 6- (red), and 8-sphere (blue) modification chains. Figure 5.4a shows that at a modification density of 5%, the pore size distribution is independent of the modification chain length. This is an interesting finding given that a system of 5% modified HMC with 4-sphere modification chains shows no signs of network formation, while the same modification density with 8-sphere modification chains forms a network containing nearly all of the chains in the system. This effect will be discussed in more detail later. Figure 5.4b shows that systems of 10% modified HMCs with 4- and 6-sphere modification chains lead to nearly identical pore size distributions, while systems with 8-sphere modification chains show a broadening of the distribution. Again it is interesting to note that the 10% modified HMC with 4-sphere modification chains did not form a stable percolated structure, while HMCs with the same modification density and 6-sphere modification chains form a network containing nearly all of the chains in the system. We do notice a broadening of the pore size distribution when the 10% modified HMC has 8-sphere modification chains, where it seems that the hydrophobic interaction becomes so strong between chains that they
are pulled closer together than in the other cases. This is supported by Figure 5.3 which shows that 10% modified HMC with 8-sphere modification chains leads to far more interactions between modification chains than all other systems tested.

The data in Figure 5.4 provides interesting insight into the importance of polymer architecture for self-assembly in solution. We observed minimal change in the pore size distributions of HMC networks when varying the modification density and modification chain length (except for 10% modified with 8-sphere modification chains). This can be explained by the comb-like architecture of the HMC. Comb copolymers can associate via the hydrophobic “teeth” of the comb rather than via the polymer backbone. Because the teeth are perpendicular to the backbone, interaction between the “teeth” can occur while the backbones remain further apart than they would in the case of a linear copolymer. This leads to a more evenly distributed system of polymer chains throughout the simulation box, and therefore a smaller pore size distribution. Our previous work, which described the effect of the degree of acetylation (DA) on pore size distributions, indicated that increasing the DA resulted in broader pore size distributions for each DA tested. The linear copolymers ended up lying flat against each other at high DA, and therefore led to the formation of larger void spaces in the system.

Next we investigated the effect of different sized hydrophobic nanoparticles on HMC network formation in solution. The concentration of hydrophobic particles was fixed at 2.0 wt% (mass of hydrophobic particles / total mass of solution (HMC + water) assuming the box is filled with water molecules), and we therefore adjusted the number of particles in the system based on the diameter, i.e. the mass of the particles. The systems with 40 Å, 60 Å,
and 80 Å diameter nanoparticles contained a total of 75, 22, and 10 nanoparticles respectively and 52 HMC chains. The concentration of HMC was 1.0 wt% in all simulations with hydrophobic nanoparticles. The packing fractions of the HMC/nanoparticle systems were approximately 0.02, while the packing fractions of the systems containing only HMC were approximately 0.005. Figure 5.5 shows snapshots at the end of simulations for systems with 8-sphere modification chains containing (a) 40 Å, (b) 60 Å, and (c) 80 Å diameter nanoparticles. These snapshots demonstrate the difference in size and quantity of nanoparticles in the systems.

Figure 5.6 shows the percolation probability of 1.0 wt% HMC solutions at a modification density of 5% and modification chain lengths of 4, 6, and 8 spheres in the presence of hydrophobic particles of diameter 40 Å, 60 Å, and 80 Å. Figure 5.6a indicates that without nanoparticles (black line), the percolation probability is approximately 0.0; this is because the system is dominated by electrostatic repulsion between the GlcN⁺ monomers. The addition of hydrophobic nanoparticles leads to a dramatic increase in the percolation probability for all nanoparticle sizes, however the presence of many small nanoparticles leads to the formation of a percolated network much more quickly than the presence of few large nanoparticles. Figure 5.6b shows that there is a low percolation probability for systems with 6-sphere modification chains in the absence of nanoparticles, while Figure 5.6c shows the formation of a stable percolated network with 8-sphere modification chains in the absence of nanoparticles. For HMCs with both 6- and 8-sphere modification chains, the addition of nanoparticles leads to a percolation probability of 1.0 regardless of the nanoparticle size, but the systems with 40 Å nanoparticles reach their equilibrium values more quickly than the
other systems. The systems with 60 Å and 80 Å nanoparticles form percolated networks at approximately the same rate.

The reason that network formation by HMCs increases in the presence of nanoparticles is that the nanoparticles act as hydrophobic junction points that promote self-assembly. Without the hydrophobic nanoparticles, the only driving force for HMC self-assembly is the attraction between the hydrophobic modification chains. When the HMC is only 5% modified, the solution behavior of HMC is dominated by electrostatic repulsion between the GlcN$^+$ monomers, especially for systems with 4- and 6-sphere modification chains. The addition of hydrophobic nanoparticles increases the hydrophobicity of the system, and gives the modification chains a greater number of favorable interaction partners. This behavior has been observed in several experimental studies showing that the addition of HMCs to blood, or solutions of nanoparticles or vesicles leads to gel formation. $^{20, 21}$ These studies show that HMCs alone do not lead to gel formation, but do form a gel in the presence of different hydrophobic solutes. Our model is able to conceptually reproduce this phenomenon. Figure 5.7 shows a zoomed in snapshot from a simulation of 8-sphere modification chain HMCs with 40 Å nanoparticles to demonstrate how the modification chains (red) interact with the nanoparticles (yellow). Blue spheres in this snapshot represent the chitosan backbone.

For all modification chain lengths tested, the HMC systems with 40 Å nanoparticles formed a percolated network earlier in time than systems with 60 Å or 80 Å nanoparticles. We believe this occurs because this system has the largest total nanoparticle surface area and a more even distribution of nanoparticles throughout the simulation box. Therefore, there is
more opportunity for the modification chains to quickly “find” a hydrophobic particle to associate with in the case of 40 Å nanoparticles than in the other cases. To understand more about how the size of a nanoparticle affects its interaction with the hydrophobic modification chains, we calculated the number of modification chains that are interacting with at least one nanoparticle throughout the simulation. Figure 5.8 shows the number of modification chains interacting with at least one nanoparticle over time, for systems with (a) 4-sphere, (b) 6-sphere, and (c) 8-sphere modification chains. The systems with 40 Å nanoparticles have more interactions between nanoparticles and modification chains than the 60 Å and 80 Å nanoparticle systems, regardless of the modification chain length. Again, we attribute this behavior to the increased surface area of nanoparticles in the 40 Å system compared to the other systems.

Next we determined how the length of the modification chains affects their ability to interact with nanoparticles of a given size. Figure 5.9 shows the number of modification chains interacting with the hydrophobic nanoparticles of varying sizes for (a) 40 Å, (b) 60 Å, and (c) 80 Å nanoparticles. Figure 5.9a shows that 4-sphere modification chains are less effective at interacting with the 40 Å nanoparticles than the 6- and 8-sphere modification chains. It is interesting to note in Figure 5.9a that the number of interactions between nanoparticles and modification chains does not change significantly when the modification chain length is increased from 6 to 8 spheres. A similar behavior is observed in Figure 5.9b, where 4-sphere modification chains lead to the fewest number of interactions between modification chains and nanoparticles, while 6- and 8-sphere modification chains show nearly identical affinity with the nanoparticles. Figure 5.9c shows very similar numbers of
interactions between modification chains and nanoparticles for each modification chain length tested.

The results in Figure 5.9 show that increasing the modification chain length does not always result in enhanced network formation over time. There appears to be a “saturation” modification chain length, 6-spheres, above which we do not see any notable increase in the number of modification chains interacting with nanoparticles. This information is useful for designing optimum HMC architectures to promote self-assembly in solution. Figures 9a-c also show that the effect of modification chain length on the number of associations between modification chains and nanoparticles becomes less significant as the size of the nanoparticles increase. We attribute this behavior to the fact that spherical nanoparticles have a decreased surface curvature with increasing diameter. Because our model does not allow the modification chains to penetrate into the nanoparticle, the only mechanism for association is adsorption to the nanoparticle surface. A decrease in the curvature allows the chitosan backbone to lie flatter against the nanoparticle than it could for a more curved surface, leading to easier adsorption of the modification chains to the nanoparticle surface. The stiffness of the chitosan backbone does not allow easy wrapping around highly curved surfaces, and therefore longer modification chains tend to be more effective for highly curved nanoparticles than shorter modification chains.

Finally we show how the addition of different sized nanoparticles affects the number of pairwise interactions between modification chains of varying length. Figure 5.10 shows the number of pairwise interactions between modification chains of length (a) 4-spheres, (b) 6-spheres, and (c) 8-spheres in the presence of 40, 60, and 80 Å diameter nanoparticles.
Figure 5.10 indicates that the presence of hydrophobic nanoparticles in the system not only causes interaction between the modification chains and the nanoparticles, but also increases the number of interactions between modification chains. We also observe that the number of pairwise interactions between modification chains is higher for systems with many small nanoparticles than for systems with few larger nanoparticles. We believe that the interactions between modification chains increases in the presence of nanoparticles because several modification chains can be attracted to one nanoparticle at the same time. This leads to a higher concentration of modification chains in the area surrounding the nanoparticles, and therefore results in increased association between the modification chains due to an avidity effect. Two modification chains can adsorb onto the surface of the nanoparticle, and freely move along the surface until they find each other. Modification chains interacting with both the nanoparticle and other modification chains result in a lower potential energy configuration than modification chains interacting only with the nanoparticle.

5.4 Discussion and Conclusions

We have presented the results of discontinuous molecular dynamics (DMD) simulations of systems containing HMCs and hydrophobic nanoparticles with the goal of understanding how hydrophobically modifying chitosan can affect self-assembly in solutions containing nanoparticles. First we investigated systems of only HMCs in solution at a concentration of 1.5 wt%. The percolation probability was calculated over time at various modification densities and modification chain lengths to determine how these parameters
control the rate of network formation, the number of interactions between chains, and the network’s pore size distribution. The results show that at a modification density of 5%, stable percolated networks are formed when using 8-sphere modification chains but not when using 4- and 6-sphere modification chains. When the modification density is increased to 10%, both 6- and 8-sphere modification chains form stable percolated networks, while 4-sphere modification chains do not.

Next we determined how the modification density and modification chain length affect the number of pairs of modification chains on different molecules that interact. Our results show that increasing the modification chain length leads to a greater number of pairwise interactions between modification chains for both 5% and 10% modified systems. Increasing the modification chain length from 6 to 8 spheres shows an approximately 350% increase in the number of pairwise interactions between modification chains. Our results are consistent with those reported by Chiu and coworkers who showed that increasing the modification density of HMC resulted in an increase in solution viscosity.\textsuperscript{18}

To understand how the choice of modification chain length and modification density affect the resulting HMC network, we calculated the pore size distribution for HMC networks with modification densities of 5% and 10% and modification chain lengths of 4, 6, and 8 spheres. The pore size distribution remained relatively constant for 5% modified HMC solutions regardless of the modification chain length. The pore size distribution for 10% modified HMC with 4- and 6-sphere modification chains was nearly identical, but a noticeable broadening of the distribution occurred when the modification chain length was increased to 8 spheres. This broadening was attributed to the significant increase in the
number of modification chain interactions that occur under these conditions, causing the chains to be pulled closer together.

Next we investigated how adding hydrophobic nanoparticles of varying sizes affected network formation in solution. Our results showed that the addition of hydrophobic nanoparticles leads to the formation of percolated HMC/nanoparticle networks for all modification chain lengths and nanoparticle diameters. This finding agrees with several previously reported experimental studies on HMC in the presence of hydrophobic solutes. For example, Chen and coworkers reported that 5% HMC with 12-carbon modification chains forms a gel in the presence of vesicles and PMMA nanoparticles but does not in their absence. Similar findings were reported by Dennis and coworkers for HMCs in the presence of carbon microspheres and by Dowling and coworkers for HMCs in the presence of blood. We also show that systems with many small nanoparticles form percolated networks earlier in time and systems with few large nanoparticles at a constant nanoparticle wt%. We believe that this is the first reported evidence of this phenomenon.

We also analyzed how the number of interactions between modification chains and nanoparticles changes with modification chain length and nanoparticle size. Our results indicate that for all modification chain lengths tested, the largest number of interactions between modification chains and nanoparticles occurs for systems with 40 Å nanoparticles, followed by the 60 Å and then 80 Å nanoparticle systems. We attribute this behavior to the fact that the total nanoparticle surface area is the largest for the 40 Å nanoparticle systems, followed by the 60 Å and 80 Å nanoparticle systems.
Finally, we calculated how the addition of hydrophobic nanoparticles to systems of HMC impacts the number of pairwise interactions between modification chains. Our data shows that the presence of nanoparticles increases the number of pairwise interactions between modification chains for all nanoparticle sizes, however this impact is most significant for systems with 40 Å nanoparticles. We believe that the nanoparticles increase the number of pairwise interactions between modification chains because two modification chains can adsorb onto the same nanoparticle, and then move freely along the nanoparticle surface until they find each other.

In conclusion we have shown that adjusting the modification chain length and modification density of HMC can be used to control network formation in solution. Additionally, we have shown that the incorporation of hydrophobic nanoparticles into the solution can have a drastic impact on self-assembly in solution, with the nanoparticles acting as hydrophobic junction points for the assembly of the modification chains. The findings in this paper are supported by previous experimental work \(^{20, 22, 26}\), and show that a simple attractive interaction between modification chains and nanoparticles can be used to study the mechanism of HMC/nanoparticle network formation. We plan to extend this model in the future to include representations for drug molecules that can be used to form an HMC hydrogel for applications in drug delivery.

Although our coarse-grained model of HMC can provide interesting insight into the mechanism of network formation, it does have several limitations. The first limitation is that the model uses an implicit solvent, meaning that molecular diffusion and hydrodynamics cannot be properly accounted for in the simulation. Another limitation is that it is difficult to
correlate the reduced time in the simulation to the real time. In order to do this, we would need to compare the reduced time to some well-defined, experimentally observed phenomenon. Finally, the interaction energy between the modification chains and the nanoparticle was not derived through a multi-scale procedure based on atomistic simulations. We used a conceptual model to capture the interaction between these two species to reflect previously observed behaviors.

5.5 Acknowledgements

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5.6 References


Figure 5.1. Coarse-grained representations of all monomer types (top) and final coarse-grained HMC representation (bottom). Colors correspond to the following groups: hydroxymethyl (red), protonated amine (blue), ring carbons (cyan), neutral amine (purple), acetyl (green), alkane (yellow). The sites of the coarse-grained HMC are to scale, however this only represents a short segment of a longer chain. Numbers refer to the coarse-grained type.
Figure 5.2. Percolation probability over time for 1.5 wt% HMC solutions with modification densities of (a) 5% and (b) 10% and modification chain lengths of 4 (black), 6 (red), and 8 (blue) spheres.
Figure 5.3. Number of pairs of modification chains that interact for 5% (black) and 10% (red) modified HMC solutions with 4-, 6-, and 8-sphere modification chains.
Figure 5.4. Pore size distributions of systems of (a) 5% and (b) 10% modified HMC with 4- (black), 6- (red), and 8-sphere (blue) modification chains.
Figure 5.5. Snapshots at the end of simulations of 1.0 wt% HMC with 8-sphere modification chains and (a) 40 Å, (b) 60 Å, and (c) 80 Å diameter nanoparticles
Figure 5.6. Percolation probability over time for 5% modified HMC with (a) 4-sphere, (b) 6-sphere, and (c) 8-sphere modification chains with and without 40, 60, and 80 Å diameter hydrophobic nanoparticles
Figure 5.7. Snapshot of 40 Å nanoparticle (yellow) interacting with 8-sphere modification chains (red). Blue spheres represent the chitosan backbone.
Figure 5.8. Number of modification chains interacting with 40 Å (black), 60 Å (red), and 80 Å (blue) hydrophobic nanoparticles for systems with (a) 4-sphere, (b) 6-sphere, and (c) 8-sphere modification chains
Figure 5.9. Number of modification chains interacting with hydrophobic nanoparticles for systems with (a) 40 Å, (b) 60 Å, and (c) 80 Å nanoparticles and modification chain lengths of 4 (black), 6 (red), and 8 (blue) spheres.
Figure 5.10. Number of pairwise interactions between modification chains for HMC with (a) 4-sphere, (b) 6-sphere, and (c) 8-sphere modification chains in the presence of 40 Å (red), 60 Å (blue), and 80 Å (pink) nanoparticles, and in the absence of nanoparticles (black).
CHAPTER 6

Conclusions and Future Work
6.1 Conclusions

In the preceding chapters, we discussed the development of a coarse-grained model of chitosan and how that model can be used to understand its behavior in aqueous solution. First we investigated how hydrophobically-modified chitosan (HMC) can be used as an oil dispersant additive to prevent oil aggregation over time. Next we developed a more detailed coarse-grained model of chitosan to predict its self-assembly behavior as a function of its degree of acetylation (DA). We further studied the effect of chitosan DA on self-assembly by investigating how the monomer sequence affects the pore size distributions of chitosan networks, and how the networks affect the diffusion of small molecules within the network. Finally we studied the self-assembly of HMC in solution with and without the presence of hydrophobic nanoparticles. Some of the key results are summarized below.

Chapter 2. A simple coarse-grained model of hydrophobically modified chitosan (HMC) was implemented to study its effectiveness as an oil dispersant additive that can prevent the formation of large oil droplets. Experimental studies have indicated that HMC could be used to delay the process of oil slick formation, but there was no previous work discussing how the modification chain length and modification density affected its ability to prevent oil aggregation. We performed simulations of HMCs with modification chain lengths of 5 and 15 spheres and modification densities of 4%, 12%, and 20% in the presence of alkanes. Our results show that HMC is effective at delaying oil aggregation for all HMC architectures tested. Long modification chains are more effective at maximizing the solvent
accessible surface area (SASA) of oil over time than short modification chains. We also find that increasing the modification density maximizes the SASA over time, but there is a saturation modification density of long modification chains above which we do not see any improved ability to prevent oil aggregation. HMCs with long modification chains disrupt the packing of oil molecules, preventing them from forming perfectly spherical droplets. Finally we show that longer modification chains lead to significantly more interactions between the HMC and oil molecules than short modification chains because of their increased ability to penetrate into oil droplets.

Chapter 3. A more detailed coarse-grained model of chitosan was developed to predict the solution behavior of chitosan as a function of the degree of acetylation (DA). The coarse-grained model contains representations for two different monomer types, protonated glucosamine (GlcN$^+$) and N-acetylglucosamine (GlcNAc). Each monomer is represented by three coarse-grained sites, resulting in a total of six unique coarse-grained types. A multi-scale modeling approach was used to develop the geometric and energetic coarse-grained parameters based on atomistic simulations. The derived parameters are able to accurately reproduce radial distribution functions, bond length fluctuations, and bond angle fluctuations observed in the atomistic simulations. The model is used to study chitosan with any DA or sequence of GlcN$^+$ and GlcNAc monomers to predict the ability of chitosan to self-assemble in solution. Our simulations focused on a random monomer sequence and DA’s ranging from 0% to 50% with a chitosan chain length of 100 monomers. Results showed that chitosan formed a percolated network in solution earlier in time with increasing DA,
indicating an increased tendency to form a gel. We observed that chitosan does not form a stable percolated network at a DA below 20%, but does at a DA above 20%. We also showed that increasing the chitosan concentration from 0.5 wt% to 2.0 wt% decreases the time required to form a stable percolated network. Finally, we showed that the sequence of GlcNAc monomers affects how many monomer associations form, with blocky sequences leading to the greatest number of monomer-monomer associations.

Chapter 4. Our coarse-grained model of chitosan was used to study the effect of GlcNAc monomer sequence on chitosan self-assembly in solution. We investigated three different monomer sequences, random, evenly-spaced, and blocky, for DA’s ranging from 10% to 50%. The results indicated that blocky sequences of GlcNAc monomers form percolated networks earlier in time than random or evenly-spaced sequences for all DA’s tested. We also show that the pore size distribution of the resulting chitosan networks changes as a function of both the DA and monomer sequence. Increasing the DA increases the average pore size of the chitosan networks, with the average pore size increasing from 4 nm to 6 nm when the DA is increased from 10% to 50%. Blocky sequences of GlcNAc monomers lead to larger pores than random and evenly spaced sequences for DAs less than or equal to 30%, while the pore size distributions for DA’s above 30% are very similar. This shows that the monomer sequence does not have a significant effect on the structure of the chitosan networks at high DA, but does have a significant impact at low DA. We showed that a network with a blocky sequence of GlcNAc monomers at 10% DA allows free diffusion of a 2 nm nanoparticle but slows the diffusion of a 6 nm nanoparticle. When the
DA is increased to 50% we see free diffusion of both 2 nm and 6 nm nanoparticles because the network has larger pores than the 10% DA network.

**Chapter 5.** We expanded our coarse-grained model of chitosan to include hydrophobic modification chains, allowing us to study the self-assembly of hydrophobically-modified chitosan (HMC) in solution with and without hydrophobic nanoparticles. HMC with 4-, 6-, and 8-sphere modification chains and modification densities of 5% and 10% were investigated. The results indicate that 5% modified HMCs form a percolated network in solution with 8-sphere modification chains, but not with 4- or 6-sphere modification chains at an HMC concentration of 1.5 wt%. When the modification density is increased to 10%, HMCs with both 6- and 8-sphere modification chains form a percolated network in solution, while 4-sphere modification chains do not. We also show that the modification chain length and modification density do not significantly affect the pore size distribution of the HMC networks, except at a modification density of 10% and a modification chain length of 8-spheres. Under the latter set of conditions we see an increase in the pore size distribution due to a significant increase in the number of interactions between modification chains. At an HMC concentration of 1.0 wt%, we see that the presence of hydrophobic nanoparticles leads to percolated HMC networks, which do not form in their absence. This occurs because the hydrophobic nanoparticles act as association sites that can connect several HMCs together. At a constant nanoparticle concentration (2.0 wt%), a system with many small nanoparticles leads to a percolated network earlier in time than a system with only a few large
nanoparticles. The presence of nanoparticles in the system also leads to a greater number of interactions between modification chains.

6.2 Future Recommendations

In this work we have discussed the development of a coarse-grained model for chitosan that can be used to predict self-assembly in solution as a function of DA, monomer sequence, and hydrophobic modification. This work has many possible future directions, some of which we recommend below.

6.2.1 Ionic Cross-Linking of Chitosan Hydrogels

One common method of chitosan network formation that we did not investigate in our work is ionic cross-linking. Chitosan is positively charged in solution at a pH less than or equal to its pKa, and therefore can be cross-linked using anionic molecules to form nanoparticles and hydrogels. Tripolyphosphatate (TPP) is a polyanion that has been commonly used to cross-link chitosan for synthesis of nanoparticles due to its high charge density.\(^1\)\(^-\)\(^3\) We believe that it would be beneficial to perform simulations of the chitosan-TPP cross-linking process to understand how the characteristics of chitosan networks such as the pore size distribution can be controlled based on the concentration of TPP. This could lead to the design of drug delivery systems that can be finely tuned by changing the concentration of TPP cross-linking agent. We will first need to develop a coarse-grained model of TPP that is compatible with our previously developed model of chitosan. We propose a 3-site coarse-
grained mapping of TPP, which is shown in Figure 6.1. Atomistic simulations of TPP with chitosan will be used to develop interaction potentials between the two molecules, and to derive the geometric constraints of the TPP molecule. After developing the coarse-grained parameters for TPP, we will perform coarse-grained DMD simulations of chitosan in the presence of different concentrations of TPP cross-linker. We will characterize the systems in a number of ways. First we will look at the time it takes for the system to form a percolated network as a function of TPP concentration, indicating the formation of a gel. Next we will analyze the pore size distribution of the networks at varying TPP concentrations and try to identify a relationship between the TPP concentration and pore size distribution, allowing us to design nanoparticles with the ability to control the release of different sized molecules.

6.2.2 pH Induced Swelling of Chitosan Hydrogels

Chitosan solution behavior is known to be strongly dependent on the solution pH. At low pH chitosan is a highly charged cationic polyelectrolyte leading to electrostatic repulsion between chains, while at neutral pH chitosan loses its positive charge leading to association of chains via hydrophobic and hydrogen bonding interactions. Previous studies have shown that lowering the pH leads to swelling of chitosan networks because more of the monomers become positively charged. We plan to investigate this phenomenon using our coarse-grained model by first running a coarse-grained simulation of chitosan at a neutral pH (uncharged monomers), and allowing the system to run to equilibrium. Then we will slowly lower the pH by randomly protonating monomers, giving them a positive charge. This will
lead to swelling of the network which we can quantify by calculating the solvent accessible surface area (SASA) and pore size distribution of the chitosan networks. These quantities will give us an approximate measure of the extent of swelling of the chitosan network as a function of the pH. We will also perform the same procedure for chitosan chains with varying degrees of acetylation and modification densities to see how the pH can be used to change the structure of the hydrogels. The fraction of protonated monomers will be related to the true pH based on previous experimental work studying net charge of chitosan as a function of pH.\(^4\) These studies could be useful for designing controlled delivery systems for cancer drugs because a pH gradient is known to occur surrounding a tumor.

6.2.3 Drug Delivery of Doxorubicin and Gemcitabine Using Chitosan Hydrogels

We would like to collaborate with Professor Stefano Menegatti at North Carolina State University to design chitosan hydrogels that can lead to controlled delivery of drugs. In particular, we will focus on the cancer drugs doxorubicin and gemcitabine, which are currently being used in a simultaneous administration protocol for cancer treatment.\(^5,6\) We will develop coarse-grained parameters for both doxorubicin and gemcitabine using the same multi-scale procedure that we used to develop parameters for chitosan and alkanes. We propose a 5-site coarse-grained mapping scheme for doxorubicin and a 2-site mapping scheme for gemcitabine, which can be seen in Figure 6.2a and 6.2b respectively. After developing the coarse-grained parameters, we will investigate how different combinations of acetylation, modification, cross-linking agents, and pH change can be used to control the
release of these drugs. Our goal is to design chitosan hydrogels that lead to simultaneous or separate release of the two drugs. Both the pore size distribution of the different chitosan networks and the intermolecular interactions between each drug with chitosan will be investigated to optimize the composition of chitosan. We will first perform DMD simulations of chitosan solutions without drugs, allowing the self-assembled networks to form. Then we will add drug molecules to the networks and measure the diffusion coefficient of these two drugs inside the chitosan networks, allowing us to predict if the drugs will release together or separately. In the future we may be able to parameterize new drugs and develop a library of drug molecules that are compatible with our coarse-grained model of chitosan. A tool such as this could lead to more efficient drug design, where our model could quickly screen many chitosan/drug combinations to determine optimum conditions for drug release.
Figure 6.1 Proposed coarse-grained mapping of tripolyphosphate (TPP)
Figure 6.2 Proposed coarse-grained mapping for (a) doxorubicin and (b) gemcitabine
6.3 References


APPENDIX
Table A1. Hard-sphere diameters and square-well/square-shoulder boundaries between each coarse-grained site. Type i and Type j refer to the coarse-grained type, # discontinuities refers to the total number of discontinuities in the interaction potential, and $\sigma_x$ refers to the separation distance corresponding to each discontinuity.

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Table A2. Interaction energies between each coarse-grained site. Type i and Type j refer to the coarse-grained type, $\varepsilon_{\text{total}}$ refers to the total number of square-wells/square-shoulders in the interaction potential, and $\varepsilon_x$ refers to the depth of each well.

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Table A3. Bond and pseudobond distances between backbone sites on chitosan (between two type 1 spheres) and chitin chains (between two type 4 spheres). Type i and Type j refer to the coarse-grained type. A bond type of “a” indicates a nearest-neighbor bond, a bond type of “b” indicates a next-nearest-neighbor pseudobond, and a bond type of “c” indicates a next-next-nearest neighbor pseudobond. The min distance is the minimum bond distance and the max distance is the maximum bond distance.

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Table A4. Bond and pseudobond distances between coarse grained sites. Type i and Type j refer to the coarse-grained type. A bond type of “a” indicates a bond, a bond type of “b” indicates a pseudobond. The min distance is the minimum bond distance and the max distance is the maximum bond distance.

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