ABSTRACT

WOODHEAD, JEFFREY LAURENCE. Computer Simulation of Drug-Encapsulating Copolymer Nanoparticles for Cancer Therapy. (Under the direction of Dr. Carol K. Hall).

Copolymer nanoparticles are being investigated as drug delivery agents, and hold great promise for increasing the solubility and improving the targeting of anti-cancer drugs. However, results from encapsulation experiments are inconsistent, and a theoretical framework for understanding drug encapsulation within block copolymer nanoparticles is necessary. Our goal is to provide some understanding of how block copolymer nanoparticles assemble in the presence of drugs and what changes in system variables improve drug encapsulation efficiency.

We perform discontinuous molecular dynamics (DMD) computer simulations on a model copolymer/drug/solvent system, where the drug is modeled as a generic solvent-phobic solute. We investigate the dependence of the structural phase behavior and encapsulation efficiency of the copolymer/solute/solvent system on the system packing fraction, the copolymer volume fraction, the head-solute interaction strength, and the head-head interaction strength. We determine what changes in these variables produce desirable solute encapsulation behavior and what changes in these variables improve the encapsulation efficiency of the system.

We find that varying our system variables produces five different structural phases. Three of these phases do not encapsulate solute: the “micelle + dispersed solute” phase, the “micelle + solute cluster” phase, and the “solute + drug cluster” phase. Two of these phases encapsulate solute: the “micelle encapsulating drug aggregate” phase and the “micelles encapsulating dispersed solute” phase. This latter phase is the ideal phase...
for effective drug encapsulation. We find that the head-solute interaction strength serves as a coupling parameter linking the copolymer and solute systems and thus dictating the encapsulating ability of the system.

We investigate the encapsulation efficiency of the system at various values of the packing fraction, polymer volume fraction, and head-head interaction strength. We find that increasing both the polymer volume fraction and the packing fraction increase the encapsulation efficiency, while increasing the head-head interaction strength decreases the encapsulation efficiency. Interestingly, both decreasing the packing fraction and increasing the head-head interaction strength make micelle formation easier while decreasing encapsulation efficiency. We discuss this result in terms of an energy barrier to drug insertion into a micelle core proposed by Kumar and Prud’homme.

We also investigate the internal structure of solute-encapsulating micelles by calculating the density profiles of the micelles. We find that increasing the packing fraction does not change the structure or size of the micelles. Increasing the head-solute interaction strength causes the solute to shift from being concentrated at the center of the micelle to being spread more evenly throughout the micelle core. We also find that increasing the head-head interaction strength causes the solute to shift from the center of the micelle onto the micelle surface.

We compare the encapsulation efficiency and solute uptake kinetics of two nanoparticle assembly methods: one in which the copolymer and solute aggregate together, and one in which the copolymer forms empty micelles and subsequently takes up solute. We find that there is no difference in encapsulation efficiency between the two
assembly methods; however, at high head-head interaction strength, uptake of solute into the previously formed micelles is significantly slower than encapsulation in the co-associating system.

Our results provide a framework for explaining and improving drug encapsulation in block copolymer micelles. We briefly discuss methods by which our research can be directly applied to actual drug-copolymer pairs, and how this could lead to improvements in drug encapsulation efficiency.
Computer Simulation of Drug-Encapsulating Copolymer Nanoparticles for Cancer Therapy

by
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DEDICATION

To William Woodhead, of blessed memory, and Selahbug. And Mom.
BIOGRAPHY

Jeff Woodhead was born in Washington, D.C. on October 7, 1981. He is the only child of William and Jan Woodhead. He is the grandson of Ira and Jean Gershner of Pine Bluff, AR and Winifred and Roy Woodhead of Tremonton, UT. He was raised in northern Virginia. He graduated from Thomas Jefferson High School for Science and Technology in Alexandria, VA in 1999 and received a full scholarship to Vanderbilt University in Nashville, TN. At Vanderbilt, Jeff co-founded and served as an editor for the Slant, a student-run humor magazine, and wrote for several other publications. He also did research into the reaction dynamics of a gas analyzer used to analyze exhaust from the campus power plant. He graduated from Vanderbilt summa cum laude with a degree in chemical engineering and history in 2003, and moved from there to graduate school at North Carolina State University. He joined Dr. Carol K. Hall’s research group in April 2005. He received his Master’s of Science in Chemical Engineering in 2006. His daughter, Selah Quinn Woodhead, was born on February 28, 2008; she has ensured that Jeff will memorize every song from the Backyardigans by the middle of 2011. For the past two years, Jeff has participated in Historic Green, a project restoring hurricane-damaged buildings in New Orleans, LA. He will begin a post-doctoral fellowship at the Hamner Institutes for Health Sciences in March 2011.
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I would like to thank my mother, Jan Woodhead, and my father, William Woodhead, for their encouragement and love. Mom has been a source of support since I first began graduate school, and indeed throughout my educational career. Dad was a great friend and mentor, and was always able to pick me up and make me laugh whenever I was discouraged. He passed away in March 2005 from lung cancer, which is a large part of the reason I selected a thesis project related to cancer research.

Finally, I would like to thank my daughter, Selah Woodhead, for keeping me grounded, reminding me that there’s more to life than graduate school, and for being so darn adorable.
TABLE OF CONTENTS

LIST OF TABLES...............................................................................................................................viii

LIST OF FIGURES..............................................................................................................................ix

Chapter 1. Introduction......................................................................................................................1
  1.1 Motivation.................................................................................................................................1
  1.2 Overview................................................................................................................................3
  1.3 References...............................................................................................................................6

Chapter 2. Computer Simulation of Micelle Formation in the Presence of Solutes...8
  2.1 Introduction..............................................................................................................................8
  2.2 Methods................................................................................................................................13
  2.3 Results and Discussion............................................................................................................20
  2.4 Conclusion.............................................................................................................................26
  2.5 Acknowledgements................................................................................................................28
  2.6 References.............................................................................................................................29

Chapter 3. Encapsulation Efficiency and Micellar Structure of Solute-Carrying
  Block Copolymer Nanoparticles......................................................................................................42
  3.1 Introduction..............................................................................................................................42
  3.2 Methods................................................................................................................................46
  3.3 Results....................................................................................................................................49
  3.4 Conclusion..............................................................................................................................56
  3.5 Acknowledgements................................................................................................................59
3.6 References .................................................................................................................. 59

Chapter 4. Future Work ................................................................................................. 76

4.1 Varying the size of the solute particle ................................................................. 77

4.2 Determining values of $\varepsilon$ for copolymer-drug pairs .................................. 78

4.3 Simulation of other polymer drug delivery vehicles ....................................... 78

4.4 Simulation of drug-carrying nanoparticles in the body .................................. 79

4.5 References .............................................................................................................. 80
LIST OF TABLES

Table 2.1  Matrix of interaction parameters..........................................................41
Table 3.1  Matrix of interaction parameters..........................................................75
LIST OF FIGURES

Figure 2.1  Size distribution function for a system that is micellar (black) and unimeric (red).................................36

Figure 2.2  An example of the “unimers + solute clusters” morphology........36

Figure 2.3  An example of the “micelles + solute clusters” morphology...........37

Figure 2.4  An example of the “micelles + dispersed solute” morphology.........37

Figure 2.5  An example of the “micelles encapsulating solute clusters” morphology..................................................................................................................38

Figure 2.6  An example of the “micelles encapsulating dispersed solute” morphology..................................................................................................................38

Figure 2.7  Phase behavior of the solute-copolymer-solvent system in the η/φ plane at $\varepsilon_{hd} = 1.0$. The grey line is the micelle/unimer transition as reported by Li and Hall.................................................................39

Figure 2.8  Phase behavior of the solute-copolymer-solvent system in the $\eta/\varepsilon_{hd}$ plane at $\varphi=0.174$................................................................................................................39

Figure 2.9  Phase behavior of the solute-copolymer-solvent system in the $\eta/\varepsilon_{hd}$ plane at $\varphi=0.244$................................................................................................................40

Figure 2.10  Phase behavior of the solute-copolymer-solvent system in the $\eta/\varepsilon_{hd}$ plane at $\varphi=0.244$................................................................................................................40

Figure 3.1  Percent of solute particles encapsulated at different system packing fractions. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\varphi = 0.174$.........................................................................................65
Figure 3.2  Percent of solute particles encapsulated at varying polymer volume fraction. \( \varepsilon_{hh} = 1.0, \varepsilon_{hd} = 1.2, \eta = 0.3 \) ..........................................................................................66

Figure 3.3  Fraction of solute particles encapsulated at varying head-head interaction strength. \( \varepsilon_{hd} = 1.2, \phi = 0.174 \) ..........................................................................................67

Figure 3.4  Number of surface-bound solutes versus packing fraction at \( \varepsilon_{hh} = 1.0, \varepsilon_{hd} = 1.2, \eta = 0.174 \) ..........................................................................................68

Figure 3.5  Number of surface-bound solute particles at varying head-head interaction strength. \( \varepsilon_{hd} = 1.2, \eta = 0.3, \phi = 0.174 \) ..........................................................................................69

Figure 3.6  Encapsulation efficiency at various values of \( \varepsilon_{hh} \) for two different means of nanoparticle assembly. \( \varepsilon_{hd} = 1.2, \eta = 0.3, \phi = 0.174 \) ..........................................................................................70

Figure 3.7  Amount of solute encapsulated versus time for two different methods of nanoparticle assembly (pre-assembled micelles and co-association) at two different values of \( \varepsilon_{hh} \). Blue: pre-assembly, \( \varepsilon_{hh} = 1.2 \). Red: co-association, \( \varepsilon_{hh} = 1.2 \). Green: pre-assembly, \( \varepsilon_{hh} = 1.5 \). Purple: co-association, \( \varepsilon_{hh} = 1.5 \). \( \varepsilon_{hd} = 1.2, \eta = 0.3 \) and \( \phi = 0.174 \) ..........................................................................................71

Figure 3.8  Density profiles for systems at a) \( \eta = 0.15 \), b) \( \eta = 0.3 \), and c) \( \eta = 0.4 \). Solute is in green, head is in black, and tail is in blue. \( \varepsilon_{hh} = 1.0, \varepsilon_{hd} = 1.2, \phi = 0.174 \) .........72

Figure 3.9  Density profiles for systems at a) \( \varepsilon_{hd} = 1.1 \), b) \( \varepsilon_{hd} = 1.3 \), c) \( \varepsilon_{hd} = 1.4 \), and d) \( \varepsilon_{hd} = 1.5, \varepsilon_{hh} = 1.2, \eta = 0.3, \phi = 0.174 \) ..........................................................................................73

Figure 3.10  Density profiles for systems at a) \( \varepsilon_{hh} = 1.2 \); b) \( \varepsilon_{hh} = 1.3 \); c) \( \varepsilon_{hh} = 1.4 \); and d) \( \varepsilon_{hh} = 1.5, \varepsilon_{hd} = 1.2, \eta = 0.1, \phi = 0.174 \) ..........................................................................................74
CHAPTER 1

Introduction

1.1 Motivation

Cancer treatment frequently involves chemotherapy, a process in which cytotoxins are introduced into the bloodstream in an attempt to kill tumor cells. While chemotherapy has had a huge positive impact on the lives of many cancer patients, it is far from being a universally useful treatment. Many chemotherapy drugs, for example paclitaxel, are poorly soluble in the bloodstream, meaning that a high dosage of drug must be introduced into the body in order to have the desired effect on the tumor. Furthermore, most current chemotherapy methods do not involve targeting the drugs so that they attack only tumor cells. This causes the drug to attack healthy cells in addition to tumor cells, which decreases the amount of drug that can be introduced into the body without life-threatening side effects. As a result, some tumors are untreatable because they would require a toxic dose of chemotherapy agent to be treated effectively.

The solubility and targeting issues can both be addressed by encapsulating drugs within nanoparticles. Encapsulating the drug within a nanoparticle can make it more soluble and can decrease the amount of drug necessary to treat tumors. Furthermore, once the drug is encapsulated, targeting ligands can be added to the surface of the nanoparticle that guide the drugs toward tumor cells and avoid the destruction of healthy non-cancerous cells.
Several varieties of nanoparticle are being investigated as possible drug delivery agents, including liposomes, diblock and triblock copolymer micelles, and highly branched dendrimers, all of which have demonstrated the ability to encapsulate drugs [1-10]. In this thesis, we will focus on drug-carrying diblock copolymer micelles.

Researchers have reported mixed results on encapsulation within diblock copolymer micelles; while some describe good encapsulation efficiency and the desired drug release profile, others have found that the encapsulation is poorer than expected and that the drug leaves the micelle too quickly [5-10]. Poor encapsulation and rapid drug release are thought to result from the drugs not having been fully encapsulated but rather remaining on the nanoparticle’s surface [5].

A theoretical approach to understanding drug encapsulation efficiency would be useful for understanding why some experiments yield good encapsulation and others do not. A mathematical model by Prud’homme and Kumar has demonstrated that drug loading reaches a maximum due to the increasing amount of energy required to insert a drug particle into the micelle core [11]. Another mathematical model by Matubayasi et al. suggests that the interaction between the solute and the micelle core is the biggest factor in determining solubilization ability [12]. Other researchers used computer simulation to study these systems; most find that changing the interaction strength between the solute and the hydrophobic part of the copolymer changes the solvation dynamics of the system [13, 14].
In this thesis we model the drug-encapsulating block copolymer system as a simple copolymer/solvent/solute model where the drug is represented by a generic solute particle. Discontinuous molecular dynamics are used to investigate the phase behavior and the encapsulation efficiency of this model copolymer-solvent-solute system. We determine which variables are most important to the system’s ability to form micelles and to encapsulate solute. We also investigate the internal structure of the micelles and the solute uptake kinetics of the system.

1.2 Overview

This section summarizes the remaining chapters in this thesis. Each chapter has its own bibliography.

In Chapter 2, we describe the phase behavior of our model system. We find that by varying the system packing fraction, the polymer volume fraction, and the head-head and head-solute interaction strengths, we can change the ability of the system to form micelles, and the ability of those micelles to encapsulate solute. We find that five different structural phases (morphologies) exist, three of which involve solute encapsulation and two of which do not. The three non-encapsulating phases are as follows: the “micelles + dispersed solute” phase, where the copolymer forms micelles but the solute does not aggregate; the “micelles + solute cluster” phase, where both the copolymer and solute aggregate but do not do so together; and the “unimers + solute cluster” phase, where only the solute aggregates. The two encapsulating phases are as follows: the “micelle encapsulating solute cluster” phase, where a loose micelle forms
around a large solute aggregate; and the “micelles encapsulating dispersed solute” phase, where solute is dispersed throughout several micelles. The two encapsulating phases are qualitatively different; the latter phase is the one that is most ideal for drug encapsulation. We conceptualize the overall system as the coexistence of two subsystems, one being the solute and the other being the copolymer. We demonstrate that as the head-solute interaction increases, these two systems become coupled, and encapsulation occurs. At the end of Chapter 2, we discuss methods that can be used for relating our system parameters to experimental data.

Chapter 3 describes our research into the encapsulation efficiency of the copolymer-solute-solvent system as well as investigations into the internal structure of the micelles and the uptake kinetics of the system. We find that the encapsulation efficiency depends strongly on polymer volume fraction, packing fraction, and head-head interaction strength. Increasing the polymer volume fraction causes the system to encapsulate more solute, most likely because more copolymer can form more micelles that can encapsulate solute. Increasing the packing fraction also increases the encapsulation efficiency of the system, while increasing the head-head interaction strength decreases the encapsulation efficiency. This implies that when micelles form more easily, less solute can become encapsulated. This result is discussed in terms of the mathematical model of drug insertion into a micelle core formulated by Kumar and Prud’homme.

We also compare the two main nanoparticle assembly methods and find that the encapsulation efficiency is the same when the copolymer and solute coassociate as when
the micelle is preassembled and subsequently loaded with solute. We do find, however, that the two solute encapsulation methods have different uptake kinetics at higher values of the head-head interaction strength. Chapter 3 also presents some of our results on the internal structure of the solute-encapsulating micelles. We find that increasing the head-solute interaction causes the solute to become distributed more evenly throughout the micelle, while increasing the head-head interaction causes the solute to shift onto the surface of the micelle.

Chapters 2 and 3 are adapted from the following publications:


1.3 References


CHAPTER 2

Computer Simulation of Micelle Formation
in the Presence of Solutes

2.1 Introduction

Two important problems associated with cancer chemotherapy are that anti-tumor drugs may be poorly soluble in the bloodstream and that they attack the body in an untargeted fashion, killing healthy cells as well as diseased cells [1,2]. Research in recent years has focused on using nanotechnology to solubilize these drugs and to make them more selective for cancer cells [3]. One way to accomplish this is to encapsulate the drugs in nanoparticles formed by block copolymer micelles that have been functionalized with targeting ligands. Encapsulation increases drugs’ solubility, and functionalization allows them to be targeted to the tumor [4]. We seek to provide a theoretical framework that describes the encapsulation of drugs within block copolymer micelles, focusing in particular on the drugs’ effect on micelle formation.

Many researchers have tried to encapsulate anti-cancer drugs in block copolymer nanoparticles, often with mixed results. Some researchers have reported success in getting a sufficient amount of drug into the core of the nanoparticle; others have reported disappointing encapsulation results, with drugs either not becoming encapsulated or remaining on the surface of the particle [5-7]. Other researchers have loaded drug or other
hydrophobic compounds into previously fabricated copolymer micelles, with similarly mixed results [8-10].

Theoretical approaches that describe micelle formation by block copolymers are numerous [11-18], but few deal specifically with the case of drug encapsulation [19,20]. One theory-based approach to drug encapsulation is that of Kumar and Prud’homme, who calculate the theoretical free energy required to place a drug molecule into an already formed micelle core. Their model contains an entropic term based on the size of the block copolymer, an enthalpic term based on the interaction between the drug and the hydrophobic micelle core, and a pressure-volume work term associated with loading the drug into the micelle core [21]. By including the PV work term, they achieve better agreement with experimental results than previous models. This model does not, however, apply to processes in which the nanoparticle assembles and encapsulates the drug simultaneously. Matubayasi et al. also explore the free energy implications of solute insertion into a micelle and determine the relative effects of solute interaction with the different portions of a micelle/solvent system. They find that interactions between either the solute or the polymer and the aqueous bulk solution have little effect on solubilization, while solute interactions with the hydrophobic core have the greatest effect [22].

While drug encapsulation specifically is not well studied in the literature, the encapsulation of cancer drugs in copolymer micelles can be seen as a specific case of the general problem of solubilization. Solubilization is the process wherein a small insoluble molecule (the “solute”) is encapsulated within a block copolymer structure in order to
increase its solubility in the solvent. A number of theoretical descriptions of solubilization phenomena in copolymer systems have appeared in the literature.

Palmer and Liu performed molecular dynamics simulations on a simple copolymer-solute system and found that changing the interaction between the solute and the copolymer could change the structure formed by the copolymer in the system. Their simulations produce micelles at low head-solute interaction strength and larger-scale composite structures at high head-solute interaction strength. They conjecture that these large composites are either the beginning of a lamellar phase or a precursor to zeolite formation. Since their simulations are geared towards the production of porous ceramics, they did not examine the encapsulation of solute within a micelle [23]. Viduna et al. examined the solubilization of short polymer chains in block copolymer systems using a lattice Monte Carlo model [24]; Patti et al. also use lattice Monte Carlo to perform simulations on their model surfactant/hybrid organic particle system [25]. Faeder and Ladanyi conducted MD simulations to determine the dynamic properties of a solute within a single surfactant micelle [26]. Both found that the interactions between the solute and the solvent-phobic portion of the copolymer change the solvation dynamics of the system. Other mathematical models of solubilization discuss the importance of the interaction between the solute and the copolymer as well, especially how solute can change the morphology of the system [27,28]. All the above studies suggest that the strength of interaction between the head and the solute affects the type of structures formed by the system.
It would be useful to understand how the characteristics of copolymer, solvent, and solute affect the encapsulation of drugs in copolymer micelles, or the solubilization of insoluble solutes in copolymer micelles. The goal would be to learn what characteristics of a copolymer are necessary for good encapsulation efficiencies. Examples of copolymer characteristics that affect micelle formation are: hydrophobicity of the copolymer head groups; the length of the copolymer; the ratio of hydrophobic head length to hydrophilic tail length; and the affinity of the hydrophobic head for the solvent. Other researchers [29-31] have found that varying these characteristics affects the ease with which micelles form, as well as the kind of micelle that is formed. Shen and Eisenberg found that by varying the solvent quality and the copolymer percentage by weight, they could create spherical micelles, rod-like micelles, or vesicles [29]. Zheng and Eisenberg found that the solvent quality is a key factor in determining whether transitions between morphologies are reversible or if the system becomes kinetically frozen in a given state. They also showed that the addition of ions to a system could make micelles form more easily [30]. Liu et al. showed that the initial copolymer concentration dictates the shape and size (and hence the solubility) of micelles formed by tri-block copolymers [31]. Missirlis et al. found that the size and hydrophobicity of the encapsulating copolymer block in triblock Pluronic copolymers also affects drug encapsulation ability, and that the solvent quality affects the ease with which the micelles form in the first place [32].

In this paper we use discontinuous molecular dynamics (DMD) computer simulations to investigate the behavior of a model copolymer-solvent system in the
presence of solute molecules. We model the copolymers as square-well chains of length 12 and the solute as square-well spheres; we then run DMD simulations until the system reaches a quasi-equilibrium state. We explore the impact that the hydrophobic solute particles have on the phase behavior of the system, including the ease with which the copolymers form micelles. We also vary the amount of copolymer in the system and the head-solute interaction strength and demonstrate how each of these variables affects the ease with which the system forms micelles and the solute encapsulation ability of the system. We also calculate the types of mesophases or morphologies that occur and plot morphological phase diagrams in the system packing fraction (η) and copolymer volume fraction (φ) plane. Finally, for selected values of φ we plot morphological phase diagrams in the plane spanned by η and the head-solvent interaction.

Highlights of our results include the following. The presence of solute particles makes micelles form more easily than they would otherwise; that is, when solute is present, copolymers in systems with fewer copolymers and higher packing fraction will form micelles when they would otherwise remain as unimers. The more strongly the solute particles interact with one another, the more the easily micelles form. We find that when strongly hydrophobic solute particles are in the system, the nature of the structures formed changes. The micelle-unimer transition that occurs in a solute-free copolymer-solvent system is often replaced by a transition between a micelle phase and a “cluster” morphology. In this cluster morphology, copolymers gather around a large aggregate of solute particles, usually composed of most of the solute particles in the system. Five morphologies were found: (1) “Unimer + solute cluster,” where micelles do not form but
solute aggregates do form; (2) “micelles + solute cluster,” in which both micelles and solute aggregates form, but the solute aggregates are not encapsulated; (3) “micelles + dispersed solute,” in which micelles form but solute remains unencapsulated and dispersed through the system; (4) “micelle encapsulating solute cluster,” wherein large solute aggregates are encapsulated by a large amount of polymer; and (5) “micelles encapsulating dispersed solute,” in which encapsulated solute is dispersed throughout numerous small micelles. This fifth morphology is ideal for drug encapsulation. The head-solute interaction strength is a key variable that, along with the packing fraction, determines whether solute is encapsulated or not. This is because the head-solute interaction acts as a coupling parameter between two coexisting systems that otherwise work independently of one another: the micelle-forming copolymers and the aggregating solutes.

The paper is organized as follows: first we present the molecular model used to describe the copolymer-solvent-solute system and the simulation method used in our investigation. We then present our simulation results and discuss their significance. Finally, we suggest how the insights gained from this work could be used in the laboratory to design more effective drug carriers.

2.2 Methods

The simulation method used in this paper is discontinuous molecular dynamics (DMD). In DMD, the interactions between molecules are described by a discontinuous potential, the square well, in contrast to traditional molecular dynamics, which uses the
Lennard-Jones potential. As a result, DMD avoids the need to integrate Newton’s laws of motion at small, regularly spaced time steps since there are only two separation distances (hard sphere diameter and square well width) at which the potential between two particles changes. Instead, particles move linearly between “collisions” that occur at the two separation distances. DMD simulation thus saves a significant amount of computational time and is well suited for investigating the dynamics of large systems over long time intervals [33]. We have chosen to use DMD because micellization and solute/drug encapsulation are relatively long-time phenomena.

DMD has been used previously by one of the authors to investigate micelle formation in the absence of solute molecules. Li and Hall explored the phase behavior of a copolymer-solvent system as a function of the system’s packing fraction, \( h \), a measure of the system density, and the block copolymer volume fraction, \( \phi \) [34]. Li and Hall’s paper was motivated by the behavior of polymers in supercritical fluids; however, it is applicable to aqueous solubilization because the range of densities of supercritical CO\(_2\) used in experiments roughly encompasses the density of water. The block copolymers were modeled as square-well chains of length twelve and the solvent molecules were modeled as square-well spheres. The number of hydrophobic “head” spheres and hydrophilic “tail” spheres per chain varied. Three distinct phases were observed: a two-phase region wherein micelles form and separate from the solvent, a one-phase (well-dissolved) micelle region, and a unimer region where the copolymers do not form micelles. Both transitions – the two phase-one phase transition and the micelle-unimer transition – occur at higher packing fractions as the mole fraction of copolymer increases.
That is, as the mole fraction of copolymer increases, a higher system density is required to solubilize the micelles and an even higher system density is required to break up the micelles.

Li et al. also looked at how the phases observed depend on the values of the interaction between hydrophobic head spheres, $\varepsilon_{hh}$, and the interaction between head and solvent spheres, $\varepsilon_{hs}$ [35]. At all packing fractions, micellization occurs at higher head-head interaction as head-solvent interaction increases. This is because head-solvent interactions discourage micelle formation by solubilizing copolymers and pulling apart copolymer aggregates; if there are more head-solvent interactions working towards solubilizing the copolymers, stronger head-head interactions are needed to form aggregates.

We have conducted DMD simulations in the canonical ensemble (constant $N$, $V$, $T$) on a copolymer-solvent-solute system. Solvent molecules (s) and solute (drug) molecules (d) are modeled as spheres of diameter $\sigma_s=\sigma_d=1.0$. (The symbol d is used for solute/drug to distinguish from s for solvent.) Copolymer molecules are modeled as chains of solvent-phobic head spheres (h) and solvent-philic tail spheres (t) of length twelve. Head and tail spheres each have diameter $\sigma_h=\sigma_t=1.0$. The copolymer model used has four head spheres and eight tail spheres ($H_4T_8$). Adjacent (“bonded”) spheres along the chain interact via a potential equal to infinity when the separation distance (r) is not between $r=(1-\delta/2)\sigma$ and $r=(1+\delta/2)\sigma$, where $\delta$ is the bond length (set to 0.1 for our model) and zero otherwise. This ensures that adjacent spheres bound to their neighbors along a chain do not detach from one another over the course of the simulation. The reduced
temperature, $T^* = kT/\varepsilon^*$ where $\varepsilon^*$ is a reference interaction strength, is decreased continuously over roughly 25000 reduced time units using the Anderson thermostat from an initial temperature of 4.0 to a final constant temperature of 1.0. This ensures that the system avoids potential kinetic energy traps. The packing fraction, a measure of how densely packed the spheres in the system are, is defined to be $\eta = 6N\sigma^3/\pi V$, where $N$ is the total number of spheres in the simulation, $V$ is the volume of the simulation box, and $\sigma$ is the diameter of all spheres in the system. In each run, $\eta$ and $N$ were specified and the desired box length, and thus $V$, was calculated from this information. The packing fraction of the system ranged from 0.15 to 0.35, the same as that considered by Li and Hall. This is because the range of densities over which supercritical CO\textsubscript{2} is investigated in the laboratory is relatively close to the density of water at room temperature. The volume fraction $\phi$ of copolymer is defined as the number of spheres of copolymer divided by the total number of spheres. It relates to the mole fraction of copolymer as follows:

$$\phi = \frac{(N_{\text{pol}} + N_{\text{drug}} + N_{\text{sol}})R}{(N_{\text{pol}}R + N_{\text{drug}} + N_{\text{sol}})x_{\text{pol}}}$$

where $x_{\text{pol}}$ is the mole fraction of polymer, $R$ is the length of the copolymer, and $N_i$ is the number of molecules of component $i$ in the system. The volume fraction of the copolymer was varied from 0.095 to 0.244; however, for the simulations where only head-head interaction strength and head-solvent interaction strength were varied, the volume fraction of copolymer was held constant at 0.175. This is a larger copolymer volume fraction than the dilute solutions used in experimental applications. This was done because the computational time required to see comparable results in a dilute
solution would have been prohibitively large. Thus we are implicitly assuming that the qualitative behavior that we have seen in concentrated solutions would also occur in dilute solutions but on a much longer time scale.

Interactions between all spheres of species i and j in the system are represented by a square-well potential, which is defined to be:

\[
U_{ij} = \begin{cases} 
\infty & \text{when } r < \sigma_{ij} \\
-\epsilon_{ij} & \text{when } \sigma_{ij} \leq r \leq (1 + \lambda_{ij})\sigma_{ij} \\
0 & \text{when } r > (1 + \lambda_{ij})\sigma_{ij} 
\end{cases}
\]

where \( r \) is the distance between two spheres, \( \epsilon_{ij} \) is the strength of the pair interaction between the two spheres (the well depth), and \( \lambda_{ij} \) is the range of interaction (the well width). As in Li and Hall’s simulations, the well width \( \lambda_{ij} \) is set to 0.75 for all interaction pairs since we assume that long-range interactions between solute and copolymers and among copolymers are negligible. Since there are four distinct sphere types in our model, there are ten pairwise interaction parameters \( (\epsilon_{ij}) \); one for each pair of components i and j. The values of \( \epsilon \) used most frequently are listed in Table 2.1. We use a hard sphere potential to describe the interactions between solvent molecules; in other words, the well depth is set to zero. The parameters \( \epsilon_{dd} \) and \( \epsilon_{hh} \) are always set to 1.0. In our discussions of how the structural phases depend on the relative hydrophobicity of the solute and copolymer head we will focus on the parameter ratio \( \epsilon_{hd}/\epsilon_{dd} \), which is equivalent to \( \epsilon_{hd}/\epsilon_{hh} \).
Since solvent molecules are, in the interest of computational efficiency, modeled as hard spheres, we do not have a direct way of modeling the hydrophobic or polar nature of the copolymer heads or tails or of the drug molecules. Instead we take an indirect approach as one would do in an implicit solvent model where hydrophobic interactions are modeled as an effective attraction between hydrophobic molecules. Thus an increase in $\varepsilon_{ij}$ associated with the head-head, head-solute, and solute-solute interaction is our way of accounting for an increase in the head or solute hydrophobicity.

Simulation details are as follows. In each simulation, particles were initially placed in a random configuration and assigned velocities according to the Boltzmann distribution at the initial reduced temperature, $T^*=4.0$. An equilibration simulation was then run for a period of 100000 reduced time units, during which the temperature of the system was quenched to a value of $T^*=1.0$. Reduced time is defined as $t^* = t\sqrt{\varepsilon^*/m\sigma}$ where $m$ is the mass of one sphere and $\varepsilon^*$ is a reference value of the well depth $\varepsilon$. A constant temperature was achieved by the use of the Anderson thermostat method involving collisions with “ghost” particles that occur at a regular time interval. The system generally reaches the reduced temperature $T^*=1.0$ after about 25000 reduced time units. The number of DMD events that occur during a certain length of time is a function of the packing fraction; over the range of packing fractions explored in this paper, 100000 reduced time units corresponds to between $5.0\times10^9$ and $9.0\times10^9$ DMD events, which requires, on average, roughly 72 hours of CPU time. Once the system reached a quasi-equilibrium characterized by little change in the system potential energy over time, a production run was then conducted for another 5000 reduced time units using the final
configuration of the equilibration run as the starting configuration. During the production run, data on solute encapsulation and on the degree of micellization in the system were recorded.

To determine whether a system displayed micelle or unimer structure, we monitored the micelle size distribution function over the course of the data production simulation. The size distribution function is calculated in the following way. First, all aggregates are identified and the number of copolymers in each aggregate is determined. An aggregate is defined to occur when the head blocks of two or more copolymers are located within 1.5σ of one another. The number of copolymers in an aggregate is called the aggregation number. A histogram of the aggregation number is collected many times over the course of the simulation. After the simulation, the histogram is averaged and normalized to represent a fraction of the total number of copolymers in the system. We follow Li et al's convention of defining a system whose size distribution function has a peak in frequency in the moderate-to-high aggregation number region (n>5) as being in the micelle phase [34]. Sample unimer and micelle size distribution functions are provided in Figure 2.1.

We also monitored the locations of the solute particles in the system over the course of the production run in order to determine whether or not they were well encapsulated. A solute particle is defined to be encapsulated if it is located within the square well of a head segment that is part of a copolymer aggregate. We also record how many solute particles are surface-bound, since researchers have attributed the poor release profiles of some drug-bearing nanoparticles to the complexation of drugs with the
surface of the micelle \([4-7]\). A surface-bound solute particle is defined as any solute particle that is encapsulated and is also within the square well of a tail sphere. We do not include such solute particles in our encapsulation data. It is important to note that solute particles “encapsulated” by small aggregates would not be encapsulated in a real system. The number of solute particles encapsulated in small aggregates is also not included in the encapsulation data. A solute particle is defined as being part of a small aggregate if it is encapsulated by a “micelle” whose copolymer aggregation number is less than six \([36-37]\).

2.3 Results and Discussion

We observed five types of mesophases or morphologies during the course of our simulation. In the first three morphologies, the solute is not encapsulated. The first morphology, shown in Figure 2.2, is the “unimers + solute cluster” morphology; it consists of a solute cluster coexisting with predominantly free chains. The second morphology, shown in Figure 2.3, is the “micelles + solute cluster” morphology; it consists of a cluster of solute molecules coexisting with copolymer micelles, but not encapsulated within the micelles. The third morphology, shown in Figure 2.4, is the “micelles + dispersed solute” morphology; it consists of non-aggregated solute coexisting with solute-free copolymer micelles.

In the fourth and fifth morphologies, the solute is encapsulated. The fourth morphology, shown in Figure 2.5, is the “micelles encapsulating solute clusters” morphology; it consists of a single large cluster that is surrounded by copolymers. The
fifth morphology, the “micelles encapsulating dispersed solute” morphology, in it the solutes are encapsulated in many different micelles and spread fairly evenly throughout the system - it is portrayed in Figure 2.6. This fifth morphology is the ideal drug encapsulating scenario.

We make distinctions among the morphologies by monitoring the percentage of solute encapsulated in the system and the size of the average solute aggregate in the system as system conditions vary. The boundary between encapsulating and non-encapsulating morphologies is located by determining the conditions at which the percentage of solutes encapsulated in the system undergoes an abrupt change. The boundary between morphologies with a solute cluster and those without a cluster is located by determining the conditions at which the size of the average solute cluster undergoes an abrupt change. In general, if the average solute cluster size is greater than half the number of solutes in the system, it is referred to as being in a “solute cluster” morphology. It is important to point out that these morphologies are not macroscopic phases, and that the transitions between them are not thermodynamic phase transitions.

We investigated the phase behavior of the system and the efficiency of solute encapsulation as a function of packing fraction ($\eta$) and polymer volume fraction ($\phi$). Recall that in Li and Hall’s study of the phase behavior of a simple copolymer-solvent solution, the system undergoes a transition from micelles to unimers as the packing fraction or density increases at constant $\phi$. The tails have more opportunity to interact with solvent molecules at high density and thus are more likely to be solubilized. This trend is also seen experimentally [34]. The introduction of solute particles into the
copolymersolvent system impacts the morphological phase behavior in two significant ways. This can be seen in Figure 2.7, which plots the transitions for both solute-free and solute-containing systems in the packing fraction-polymer volume fraction plane. The gray line represents Li and Hall’s solute-free curve, while the black line represents our data using the interaction parameters listed in Table 2.1. First, we notice that when solute is added, the transition shifts markedly to a higher packing fraction, meaning that micelles form more easily and are more stable when solute is present. A likely explanation for this is that the hydrophobic solute particles are holding the micelles together to some extent. Second, the micelle-unimer transition that is present in the absence of solute molecules is replaced by a transition from the “micelles encapsulating dispersed solute” morphology to the “micelles encapsulating solute clusters” morphology. Even increasing the packing fraction beyond the range (0.2 to 0.35) explored by Li and Hall does not lead to a transition to a unimer phase. Clearly, the solute’s interaction with the copolymer has a profound effect on the structural phase behavior of the system.

To better understand the effect of the solute molecules on the structural phase behavior, we considered how the type of morphologies observed would change as the packing fraction and the head-solute interaction strength, represented by the ratio $\varepsilon_{hd}/\varepsilon_{hh}$, were varied, holding the polymer volume fraction ($\phi_{pol}$) constant at 0.174. Recall that since $\varepsilon_{dd}$ and $\varepsilon_{hh}$ are set to 1.0, $\varepsilon_{hd}/\varepsilon_{hh}$ is equivalent to $\varepsilon_{hd}/\varepsilon_{dd}$. Furthermore, since the Flory Chi parameter $\chi_{hd} \sim \varepsilon_{hd} - (\varepsilon_{dd} + \varepsilon_{hh})/2$, then $\varepsilon_{hd}/\varepsilon_{hh} \sim \chi_{hd} + 1$. The result is plotted in Figure 2.8. Error bars (not shown) on the phase boundaries are estimated to be ±0.05 on
the $\epsilon$-axis and $\pm 0.01$ on the $\eta$-axis. We immediately notice the distinction between the region of the graph characterized by a low $\epsilon_{hd}/\epsilon_{hh}$ and that characterized by a high $\epsilon_{hd}/\epsilon_{hh}$.

In the region where the head-solute interaction strength is low, an increase in the packing fraction (compression) leads to a change in the system from the “micelles + dispersed solute” morphology to the “micelles + solute clusters” morphology, and then upon further compression transitions into the “unimers + solute clusters” morphology. This is consistent with our earlier statement that compressing the system generally causes micelles to fall apart. Furthermore, system compression would also encourage the formation of solute aggregates due to the attractive solute-solute interaction. What we see is that during compression in the low $\epsilon_{hd}/\epsilon_{hh}$ regime, micelle dissociation and solute aggregate formation act independently of one another. We also note that the system does not encapsulate solute at low $\epsilon_{hd}/\epsilon_{hh}$. In the absence of head-solute interaction, the solute and the copolymers act as separate systems; the solute aggregates at high packing fraction due to high solute-solute interaction and the copolymers dissolve at high packing fraction due to the tail-solvent interaction. These two processes do not affect each other.

As the interaction between solute and copolymer, as measured by $\epsilon_{hd}/\epsilon_{hh}$, increases, the solute system and the copolymer system begin to affect each other. In this regime, encapsulation and the commingling of solute and copolymer aggregates becomes the norm. In our model, the system encapsulates solute at all values of $\eta$ when $\epsilon_{hd}/\epsilon_{hh}$ is larger than 0.8. Furthermore, when $\eta$ is high we see the appearance of the “micelle encapsulating solute clusters” morphology when $\epsilon_{hd}/\epsilon_{hh}$ is close to the encapsulation boundary value of 0.8. The range of interactions over which this morphology occurs is
smaller at lower packing fractions, and the morphology does not occur when the system packing fraction is extremely low. As \( \frac{\varepsilon_{hd}}{\varepsilon_{hh}} \) increases past 1.10, the system is always in the “micelles encapsulating dispersed solute” morphology, the metaphase with the best encapsulation behavior. It is remarkable that the “micelles encapsulating dispersed solute” morphology occurs even at the highest packing fraction investigated, suggesting that the strong interaction between solute molecules and the copolymer head segments pulls micelles together despite the fact that the copolymer alone will not aggregate at this packing fraction.

We also investigated the structural phase behavior of the system in the \((\eta, \frac{\varepsilon_{hd}}{\varepsilon_{hh}})\) plane at different polymer volume fractions, \(\phi_{pol}\). These results can be seen in Figures 2.9 and 2.10. When we compare the case where the polymer volume fraction is high, \(\phi_{pol} = 0.244\), (Figure 2.9) to our base case, \(\phi_{pol} = 0.174\) (Figure 2.8), we observe two main changes. The first is that the boundary between the “micelles + solute clusters” and the “unimers + solute clusters” morphologies moves to a higher packing fraction. The second change is that the “micelle encapsulating solute clusters” morphology vanishes at a higher packing fraction than it does in the base case. In the no-solute system, an increase in the packing fraction causes the micelle/unimer transition to shift to a higher packing fraction. Since copolymer and solute are acting separately in the range of \(\frac{\varepsilon_{hd}}{\varepsilon_{hh}}\) where these morphologies are present, it is therefore reasonable to expect the change in the polymer volume fraction to increase the packing fraction at which the micelle/unimer transition occurs.
The situation is quite different when we look at the case where the polymer volume fraction is low compared to the base ($\phi_{pol} = 0.098$, Figure 2.10). At this polymer volume fraction, the “micelle encapsulating solute clusters” morphology vanishes at a far lower packing fraction than in the base case and spans a somewhat wider range of $\varepsilon_{hd}/\varepsilon_{hh}$ values at high packing fraction. Furthermore, the “micelles + solute clusters” morphology has disappeared, since the micelle/unimer transition is occurring at the same packing fraction as the solute’s disperse/cluster transition. Again, this is due to the fact that at low $\varepsilon_{hd}/\varepsilon_{hh}$ the copolymer and solute systems are acting separately, so the micelle/unimer transition will shift to a lower packing fraction just as it does in the no-solute case. In this case, it has decreased to below the packing fraction above which the solute forms clusters, so solute clusters and micelles do not coexist independently.

The change in the size of the “micelle encapsulating solute clusters” morphology as the polymer volume fraction is varied suggests that this morphology is indicative of weak interaction between the solute and the copolymer systems. That is, at the intermediate values of $\varepsilon_{hd}/\varepsilon_{hh}$ at which the “micelles encapsulating solute clusters” morphology is present, the copolymer head block and the solute work together enough to form micelles that encapsulate solute, but not enough to spread the drug out throughout the system. Rather, the drive of the solute to aggregate overpowers the solute-head interaction and causes large aggregates to form. As $\varepsilon_{hd}/\varepsilon_{hh}$ increases, the head-solute interaction is able to take over and disperse the solute throughout the system.
2.4 Conclusions

We have demonstrated that the presence of solute molecules can “seed” the formation of micellar nanoparticles and make these nanoparticles easier to form. The changes in the phase diagram in the \((\phi, \eta)\) plane when solute is added indicate that the solute is clearly pulling copolymers together and keeping them together when they would otherwise not associate.

We have shown that five different morphologies form as a result of the mixing of hydrophobic solute particles and amphiphilic block copolymers. Depending on the strength of the interaction between hydrophobic head block and the solute and the packing fraction of the system, the system might be in a state where micelles and dispersed solute coexist independently, where unimers and solute clusters coexist independently, where micelles and drug clusters coexist independently, where micelles encapsulate large solute clusters, or where several micelles encapsulate several smaller solute aggregates. It is this last morphology that would be considered ideal drug encapsulation in a laboratory setting. Experimentalists may not be able to vary the packing fraction in a drug encapsulation system because most assemblies occur in an aqueous system; however, it is worth noting that the ideal drug encapsulation morphology occurs at all packing fractions investigated.

We have also demonstrated that the attraction of the head block to the hydrophobic solute particle is the most important determinant of the phase behavior of the system. High \(\varepsilon_{\text{hd}}/\varepsilon_{\text{nh}}\) causes the system to encapsulate well; low \(\varepsilon_{\text{hd}}/\varepsilon_{\text{nh}}\) causes encapsulation to be poor or to not occur at all. Encapsulation occurs at packing fractions
and volume fractions that would not normally be associated with micellization or square-well particle aggregation, suggesting that the solute-head interaction is truly driving the process.

We are left, then, with the question of how to relate our findings on the effect of $\varepsilon_{\text{hd}}$ on the phase behavior of the copolymer/solvent/solute system to experimental conditions. The interaction parameter $\varepsilon_{\text{hd}}/\varepsilon_{\text{hh}}$ is a measure of the attraction between the head block of the copolymer and the solute; as such, changing $\varepsilon_{\text{hd}}/\varepsilon_{\text{hh}}$ involves changing either the head block or the solute, or both. It would be useful to understand how one might measure and change $\varepsilon_{\text{hd}}$ so that encapsulation occurs.

There are several methods that could be used to estimate or measure $\varepsilon_{\text{hd}}/\varepsilon_{\text{hh}}$, or equivalently $\chi_{\text{hd}}$, the Flory-Huggins effective interaction parameter. One method involves the use of inverse gas chromatography to measure the interaction between a polymer immobilized in a column and a solute that flows through that column. Milczewska and Voelkel used this method to determine the strength of the interaction between a polymer and an inorganic filler [38]. Another way to extract these parameters is to use the Cosmo-SAC approach of Lin and Sandler to predict the octanol-water partition coefficients of the head and drug molecule. This approach uses approximations for the contribution to the partition coefficient from various functional groups to determine the overall partition coefficient for the molecule [39]. Computer simulations have also been used to calculate the interaction strength between two molecules; Koishi et al. used atomistic simulation to calculate hydrophobic interaction strength [40]. This method could also be applied to copolymer head blocks and drug/solute molecules. Lacombe and Sanchez have used the
Ising equation of state to calculate the interaction energy between several common molecules and generated tables of interaction energies [41, 42]. Sanchez and Lacombe also expanded the Ising equation of state for use on polymers, and calculated the interaction energies between isobutylene and several common organic solvents [43].

The methods mentioned above could be applied to the case of drug encapsulation as well. Forgacs and Cserhati generated a table of the interaction strengths between a polymer of beta-cyclodextrin and various steroidal drugs by using reverse-phase thin layer chromatography with methanol as the organic solvent. They found a large variance in the interaction strength associated with relatively minor changes in the drug structure [44]. Also, Miyajima et al. determined the partition coefficients of various drugs in water and poly(L-lactic acid) [45]; the partition coefficient has been used to calculate the interaction parameters by others [46, 47]. These methods could be applied to other drug-polymer pairs in order to test for a good interaction strength that would lead to good encapsulation.

### 2.5 Acknowledgements

We would like to thank the Graduate Assistance in Areas of National Need (GAANN) fellowship for the funding for this project, and we would also like to thank Dr. Robert Prud’homme for many enlightening conversations. We would also like to thank Dr. Steven Smith, Dr. Andrew Schultz, and Dr. Zhengmin Li for providing us with their simulation code.
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2.7 List of Tables

Table 2.1   Matrix of Interaction Parameters..........................................................41
2.8 List of Figures

Figure 2.1  Size distribution function for a system that is micellar (black) and unimeric (red)......................................................................................................................36

Figure 2.2  An example of the “unimers + solute clusters” morphology......................36

Figure 2.3  An example of the “micelles + solute clusters” morphology.....................37

Figure 2.4  An example of the “micelles + dispersed solute” morphology...............37

Figure 2.5  An example of the “micelles encapsulating solute clusters” morphology.................................................................................................................................38

Figure 2.6  An example of the “micelles encapsulating dispersed solute” morphology.................................................................................................................................38

Figure 2.7  Phase behavior of the solute-copolymer-solvent system in the η/ϕ plane at $\varepsilon_{bd} = 1.0$. The grey line is the micelle/unimer transition as reported by Li and Hall.................................................................................................................................39

Figure 2.8  Phase behavior of the solute-copolymer-solvent system in the η/ε_{bd} plane at $\phi=0.174$.................................................................................................................................39

Figure 2.9  Phase behavior of the solute-copolymer-solvent system in the η/ε_{bd} plane at $\phi=0.244$.................................................................................................................................40

Figure 2.10 Phase behavior of the solute-copolymer-solvent system in the η/ε_{bd} plane at $\phi=0.244$.................................................................................................................................41
Figure 2.1. Size distribution function for a system that is micellar (black) and unimeric (red).

Figure 2.2. An example of the “unimers + solute clusters” morphology.
Figure 2.3. An example of the “micelles + solute clusters” morphology.

Figure 2.4. An example of the “micelles + dispersed solute” morphology.
Figure 2.5. An example of the “micelles encapsulating solute clusters” morphology.

Figure 2.6. An example of the “micelles encapsulating dispersed solute” morphology.
Figure 2.7. Phase behavior of the solute-copolymer-solvent system in the $\eta/\phi$ plane at $\varepsilon_{hd} = 1.0$. The grey line is the micelle/unimer transition as reported by Li and Hall.

Figure 2.8. Phase behavior of the solute-copolymer-solvent system in the $\eta/\varepsilon_{hd}$ plane at $\phi=0.174$. 
Figure 2.9. Phase behavior of the solute-copolymer-solvent system in the $\eta/\varepsilon_{\text{hd}}$ plane at $\phi=0.244$. 
Table 1.1. Matrix of interaction parameters.

<table>
<thead>
<tr>
<th>$\varepsilon_{ij}$</th>
<th>Head</th>
<th>Tail</th>
<th>Solvent</th>
<th>Solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
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<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
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<tr>
<td>Solvent</td>
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<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Solute</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
CHAPTER 3

Encapsulation Efficiency and Micellar Structure of Solute-Carrying Block Copolymer Nanoparticles

3.1 Introduction

Nanoparticles formed by the micellization of block copolymers in the presence of cancer drugs are now being investigated as a way to reduce the low drug solubility and imprecise targeting associated with standard chemotherapy [1,2]. For example, the ability of micelles formed by PEG-b-poly(propylene sulfide) to encapsulate the water-insoluble drug cyclosporin A is the subject of a report by Velluto et al [3]. Encapsulating a drug within a micelle increases the drug’s effective solubility in the bloodstream, allowing smaller doses to be delivered with the same effect. Targeting ligands added to the nanoparticle surface increase the likelihood that the drug will be released in the vicinity of cancer cells, and prevents them from killing healthy cells [2]. Researchers studying these types of drug delivery systems have, however, reported some problems, most notably with low encapsulation efficiencies and undesirably fast release profiles [4-7].

The aim of our work is to provide a theoretical framework that describes the assembly of block copolymer micelles in the presence of drug molecules. Motivation for this work is our hope that this will serve as a guide that can assist researchers on the front line to optimize the drug encapsulation efficiency of their systems. Computer simulation can be used to provide this theoretical framework. Although other researchers have used computer simulation, as well as theoretical methods, to study micellization of block
copolymers [8-15], none of them specifically address what occurs when drugs are introduced into a block copolymer system. This is the gap that we seek to fill.

Prud’homme and Kumar formulated a theory of drug encapsulation that describes the free energy necessary to insert a drug into the core of an already-formed block copolymer micelle [16]. They determined that the free energy needed to insert a drug into the core of the micelle increases with the number of drugs in the micelle until the system reaches equilibrium and no more drug can be inserted. Their model describes the case in which micelles form before drug is introduced into the system and hence does not apply to the case considered here in which the micelle-forming block copolymer and the drug are introduced into solution simultaneously.

In a previous study [17], we modeled drug encapsulation as a solubilization process wherein generalized model solutes are encapsulated in a block copolymer micelle immersed in a solvent. Discontinuous molecular dynamics computer simulations were used to investigate the structural phase behavior of this model copolymer-solute-solvent system to determine: (a) the effect of hydrophobic solute particles on the phase behavior of the system, and (b) which system variables most affect that phase behavior. We found that the presence of hydrophobic solute particles makes micelles form more easily, and that the key variable that determines whether the system encapsulates solute well is the head-solute interaction strength ($\varepsilon_{hd}$). The copolymer/solute/solvent system formed five types of structural phases or morphologies. In three of these phases, all of which occur at low values of $\varepsilon_{hd}$, the solute and copolymers act as coexisting separate systems: micelles may form while the solute remains unaggregated, the solute may aggregate outside the
micelles, or the solute may aggregate while the polymers do not. In the other two phases, encapsulation occurs, but the manner in which the solute is encapsulated is significantly different. In the first of these phases, which occurs at moderate values of $\epsilon_{\text{hd}}$ and at high packing fraction, the solute particles form a large cluster that is surrounded by copolymer. In the second phase, which occurs at high $\epsilon_{\text{hd}}$ and all packing fractions, the micelles formed are much smaller and the solute is evenly dispersed among them. This phase, which we referred to as “micelles encapsulating dispersed solute,” is characteristic of the kind of drug encapsulation sought in the lab.

In this paper we focus in on the encapsulation of drug (solute) molecules in the “micelles encapsulating dispersed solute” phase described in our previous paper on copolymer/solute/solvent systems. Discontinuous molecular dynamics simulations are applied to this three-component system (copolymer, drug, solvent) with the copolymers modeled as square-well chains of length 12 and the drugs modeled as square-well spheres. The dependence of the encapsulation efficiency and micelle density profile on the packing fraction, polymer volume fraction, head-solute interaction strength, and head-head interaction strength is analyzed. Our results are compared to the predictions of the Prud’homme-Kumar model so as to determine the degree to which their model can be applied to systems where copolymer and solute co-associate. We also model the encapsulation efficiency of a system where the micelles form before the solute is introduced in order to compare the encapsulation efficiency and kinetics of these two methods of nanoparticle construction.
Highlights of our results include the following. Increasing the system packing fraction increases the amount of solute encapsulated and decreases the amount of solute that remains on the micelle surface. Increasing the polymer volume fraction increases the encapsulation efficiency of the system as expected, but this effect saturates at high polymer volume fraction. The encapsulation efficiency of the system depends quite sensitively on the values of the interaction parameters, especially the head-head interaction strength. Increases in the head-head interaction strength strongly impede encapsulation ability by forcing drug particles to remain on the micelle surface. Analysis of the density profiles of the solute-encapsulating micelles indicates that increases in head-solute interaction strength lead the solute and head blocks to be distributed more evenly throughout the core of the micelle, while an increase in head-head interaction strength causes micelles to become smaller and solute to shift from the center of the micelle to the outside. There is surprisingly little difference in encapsulation efficiency between the case in which solutes are introduced to a pre-formed system of micelles and the case in which solutes and copolymer co-associate. There is, however, a difference in the kinetics of encapsulation between the two nanoparticle assembly methods at high values of the head-head interaction strength. Solute uptake for the case in which micelles co-associate with copolymer is faster than in the case in which micelles are pre-formed; however, both cases result in the same overall encapsulation efficiency. Our results can be interpreted in term of the model of Kumar and Prud’homme; as conditions become more conducive to micelle formation, a stronger energy barrier to solute insertion forms which in turn decreases the encapsulation efficiency of the system.
3.2 Methods

As in our previous research, we used discontinuous molecular dynamics (DMD) simulation. In DMD, particle interactions are modeled by square-well potentials instead of by Lennard-Jones potentials as in traditional molecular dynamics simulations, eliminating the need to numerically integrate Newton’s laws at discrete time steps. This saves computational time, making DMD well suited to modeling large systems over long time periods [18]. Since the encapsulation of solute within micelles occurs over a long time and requires a large system to investigate properly, DMD is ideal for our purposes.

The model used in this paper is the same model as that employed in our previous paper on micelle formation in the presence of solutes [17]. The copolymer is modeled as a chain of twelve square-well spheres with four solvent-phobic head (H) spheres and eight solvent-philic tail (T) spheres, represented symbolically as $H_4T_8$. Solute particles (D) are modeled as square-well spheres. Solvent molecules (S) are modeled as single spheres with square-well interactions with head and tail spheres and hard-sphere interactions with other solvent spheres. All spheres have a diameter of $\sigma=1.0$. The depth of the square well potential between two particles, $\varepsilon_{ij}$, is a measure of the strength of the interaction between them. The values for the interaction energies in the model are given in Table 3.1; the head-solute, $\varepsilon_{hn}$, and head-head interaction, $\varepsilon_{hh}$, strengths are varied.

Other system variables are as follows. The packing fraction, which is related to the density of the system, is defined as $\eta=6N\sigma^3/\pi V$, where $N$ is the number of spheres in
the system and V is the volume of the simulation box. The polymer volume fraction is the number of polymer spheres divided by the total number of spheres, and is related to the polymer mole fraction, \(x_{pol}\), as follows:

\[
\phi = \frac{(N_{pol} + N_{drug} + N_{sol})R}{(N_{pol}R + N_{drug} + N_{sol})x_{pol}}
\]

where \(R\) is the length of the copolymer, and \(N_i\) is the number of spheres of component \(i\). The reduced temperature is defined as \(T^*=kT/\epsilon^*\), where \(k\) is Boltzmann’s constant and \(\epsilon^*\) is a reference interaction energy.

We have maintained our system variables so that they fall in the “micelles encapsulating dispersed solute” portion of the phase diagram from our previous research\(^\text{17}\). In this phase, the copolymers form numerous moderately sized micelles with the solute distributed evenly among them. The values for the variables are: packing fraction \(\eta = 0.3\), polymer volume fraction \(\phi = 0.174\), and solute mole fraction of \(x_d = 0.0174\). The head-solute interaction strength (\(\epsilon_{hd}\)) and head-head interaction strength (\(\epsilon_{hh}\)) are variable. The reduced temperature \(T^*\) is held constant at 1.0 after a steady cooling from an initial temperature of \(T^* = 4.0\); this is done to ensure that the system is well-mixed and to prevent it from becoming trapped in a local energy minimum. The temperature is kept constant through the use of the Anderson thermostat wherein “ghost particles” collide with system particles regularly in order to keep the velocities of the particles consistent with the Boltzmann distribution at a given temperature [19,20]. The solute volume fraction and the polymer volume fraction are higher than the values generally used in experiments. These values were chosen for computational convenience:
simulations of systems with smaller, more-realistic values take much too long to equilibrate. We do not expect that the high volume fraction and mole fraction used in our research will have a significant qualitative effect on our results regarding other variables.

DMD simulations are performed in the canonical ensemble (constant N, V, T). The number of particles in the system is held constant at 13683, with 2400 copolymer spheres configured in 200 H₄T₈ chains, 200 solute spheres, and 11083 solvent spheres. The number of copolymers in the system changes when the polymer volume fraction changes; in order to hold the drug mole fraction constant, we must change the number of solvent spheres in the system when this occurs.

Each data point requires an equilibration run and a production (data collection) run. In an equilibration run, the initial configuration is a system of randomly placed H₄T₈ chains in random coil conformations, solvent molecules, and drug molecules. The simulation proceeds for a total of 100000 reduced time units (roughly 4x10⁹ DMD events) though this depends upon the system size and packing fraction. The final configuration is then used as the initial configuration for a 5000-time unit production run during which the number of encapsulated drugs in each system and the density profile of each micelle is recorded.

The encapsulation efficiency and related terms are defined in the following way. A solute particle is defined to be “encapsulated” when it is within the square well of a head sphere that is part of a micelle containing six or more copolymers, but is not on the surface of the micelle, meaning it is not also within the square well of a tail sphere. A solute particle is defined to be “surface-bound” when it within the square well of a tail
sphere that is part of a micelle containing six or more copolymers and also within the square well of a head sphere. Surface-bound drug molecules have been implicated in poor release patterns of drug-carrying nanoparticles [4,19,20]. We restrict our attention to micelles with copolymer aggregation number of 6 or greater because aggregates with aggregation number less than 6 have also been ignored by previous researchers [21,22]. We define encapsulation efficiency as the percentage of solute spheres in the system that are encapsulated.

The density profile of any component across a solute/copolymer aggregate is determined by finding the aggregate’s center of mass, calculating the number of component spheres in a spherical shell at each distance from the center of mass and then dividing by the volume of the shell.

### 3.3 Results

We first investigated the effect that variations in the packing fraction and polymer volume fraction had on the encapsulation efficiency of the system. Figure 3.1 shows the percentage of solutes encapsulated as a function of the system packing fraction at \( \varepsilon_{hd} = 1.2, \varepsilon_{hh} = 1.0, \) and \( \phi = 0.174 \). As the graph shows, the encapsulation efficiency of the system increases moderately as the packing fraction increases. This is due to the fact that increases in packing fraction make it harder for micelles to form. As the Kumar-Prud’homme model suggests [16], the harder it is for micelles to form, the lower the energy barrier to solute entry into the micelle, thus enhancing the amount of solute encapsulated within the micelle. We will explore this phenomenon in more depth shortly.
Figure 3.2, which shows encapsulation efficiency as a function of the polymer volume fraction at $\varepsilon_{hd} = 1.2$, $\varepsilon_{hh} = 1.0$, and $\eta = 0.3$ demonstrates that increases in polymer volume fraction increase the encapsulation efficiency of the system. The existence of more polymer in the system leads to more micelles and thus more encapsulation. The effect of polymer volume fraction on encapsulation efficiency is not linear but instead levels off at $\phi$ greater than 0.175. This is likely due to the fact that at the higher polymer volume fractions, we are left with excess polymer.

It is interesting to examine how the chemical nature of the copolymers and solutes affects the encapsulation efficiency. We use the interaction parameters as a proxy for (or measure of) the type of copolymer or drug molecule considered, focusing on the head-head interaction strength, $\varepsilon_{hh}$, and the head-solute interaction strength, $\varepsilon_{hd}$. While determining specific values of $\varepsilon_{hh}$ and $\varepsilon_{hd}$ are beyond the scope of this paper, our previous paper contains a discussion of how these parameters relate to the type of polymer and drug in the system and how one might calculate these parameters for a particular copolymer-solute pair [17]. A change in $\varepsilon_{hh}$ is indicative of a change in copolymer hydrophobicity – a higher value of $\varepsilon_{hh}$ means that the head block of the copolymer is more hydrophobic. Meanwhile, a change in $\varepsilon_{hd}$ is indicative of increased or decreased affinity of the polymer head for the solute.

Figure 3.3 shows how the encapsulation efficiency (percentage of solute encapsulated) at $\phi = 0.174$ and $\varepsilon_{hd} = 1.2$ varies with $\varepsilon_{hh}$ at three different packing fractions: $\eta = 0.1, 0.2,$ and $0.3$. We examined values of $\varepsilon_{hh}$ from 0.8 to 1.5; lower values of $\varepsilon_{hh}$ didn’t result in micelle formation. At $\eta = 0.3$, the encapsulation efficiency is
constant at nearly 90% as $\varepsilon_{hh}$ increases from 0.8 to 1.1 but declines steeply thereafter, reaching an encapsulation efficiency of less than 50% at $\varepsilon_{hh} = 1.5$. Thus, increasing the head blocks’ self-affinity hinders the encapsulation efficiency of the system. A plausible explanation for this is that the attraction between the head blocks creates a high energetic barrier to entry into the micelle core, so that solute particles that would normally enter the micelle core get trapped on the surface. Thus, even though the micelles form more easily as $\varepsilon_{hh}$ increases, they form so tightly that they lock drugs out.

Figure 3.3 also shows how the packing fraction influences the dependence of encapsulation efficiency on $\varepsilon_{hh}$. At low $\varepsilon_{hh}$, the packing fraction has little effect on the encapsulation efficiency of the system, and the three curves for $\eta = 0.1$, 0.2, and 0.3 overlap. However, as $\varepsilon_{hh}$ increases, the curves separate; the encapsulation diminishing effect of increasing $\varepsilon_{hh}$ is far more pronounced at the lower packing fractions. At the highest value of $\varepsilon_{hh}$, the difference is stark; at $\eta = 0.3$, the system still encapsulates over half the solute in the system, while at $\eta = 0.1$, the system encapsulates roughly 10% of the available solute. These results are consistent with our earlier results that show that encapsulation efficiency increases as packing fraction increases.

The connection between the conditions that encourage the formation of micelles and the likelihood that a solute will be surface-bound can be seen in Figures 3.4 and 3.5. Figure 3.4 shows the number of surface bound solutes as a function of packing fraction when $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$ and $\phi = 0.174$. The number of surface-bound solutes decreases as packing fraction increases. Figure 3.5 shows the number of surface bound solutes as a function of the head-head interaction strength, $\varepsilon_{hh}$, at $\eta = 0.3$, $\phi = 0.174$ and $\varepsilon_{hd} = 1.2$. As
head-head interaction strength increases, more solute becomes bound to the surface of the micelle. Since both increasing the head-head interaction strength and decreasing the packing fraction make micelle formation easier, this reveals that increasing the tendency of copolymers to form micelles increases the number of solute particles on the surface of the micelle while decreasing the amount of solute encapsulated within the micelle.

It is of interest to relate our findings to the theory of drug encapsulation in previously formed micelles introduced by Kumar and Prud’homme. They derive an expression for the change in the surface energy of a micelle when solute is added to the micelle core and discuss how this energy barrier is perturbed by the encapsulation of additional solutes. As the drug loading increases, the free energy required to load a drug into the particle increases until an equilibrium solute loading value is reached. After this equilibrium value is reached, the drug particles do not possess the energy required to enter the micelle core, and loading stops [16]. This theory can be used to explain the results of our simulations. In our simulations, the amount of surface-bound solute increases as encapsulation becomes less likely. Solute particles become stranded on the micelle surface; a plausible explanation for this is that the solute particles that are attempting to enter the micelle core lack the energy to do so. Furthermore, our results show that as the ease of forming micelles increases, encapsulation of solute becomes less likely; this implies that the energy barrier to solute entry grows stronger when micelle formation becomes more favored. This is why changes in system variables that make micelle formation easier – increasing $\eta$ and increasing $\epsilon_{hh}$ – decrease the encapsulation efficiency of the system while increasing the amount of solute on the micelle surface.
It is of interest to see how the nanoparticle assembly method – co-association of micelles and solute or introduction of solute into pre-formed micelles – affects the encapsulation efficiency. The simulations described in Figure 3.3 at $\eta=0.3$ were re-run for the case in which solutes were added to a system of pre-formed micelles at $\phi = 0.174$ and $\epsilon_{hd} = 1.2$. A solute-free system of $\text{H}_4\text{T}_8$ molecules in an initially random configuration was allowed to equilibrate for 100000 reduced time units resulting in the formation of micelles. Solutes were then introduced, and the resulting system was equilibrated for another 100000 reduced time units. Figure 3.6 compares the encapsulation efficiency as a function of $\epsilon_{hh}$ in both cases. We can see that as we vary $\epsilon_{hh}$, there is essentially no difference between the case where solute is loaded with the copolymer and the case in which the solute is loaded after micellization. This shows that the strength of the energy barrier does not depend on the method of nanoparticle assembly, and the encapsulation efficiency at which the barrier becomes too difficult for solute to overcome is determined thermodynamically, as Kumar and Prud’homme predicted.

We also compared the solute uptake kinetics for the two nanoparticle assembly methods. Figure 3.7 shows the amount of solute encapsulated in micelles at $\epsilon_{hd} = 1.2$, $\eta = 0.3$ and $\phi = 0.174$ for both pre-assembled micelles and co-association over the first 1000 reduced time units for $\epsilon_{hh} = 1.2$ and $\epsilon_{hh} = 1.5$. At $\epsilon_{hh} = 1.2$, the initial slopes of the curves for both assembly methods are the same, meaning that the initial rate of uptake into micelles does not depend strongly on the construction method. At $\epsilon_{hh} = 1.5$, however, there is a marked difference in the initial uptake rate. For the first 300 reduced time units,
micelle uptake into pre-assembled micelles is significantly slower than encapsulation by coassociation.

We also investigate the manner in which the uptake kinetics of the two assembly methods changes when $\varepsilon_{hh}$ is increased. In the co-association case, the rate of solute uptake is roughly the same for both values of $\varepsilon_{hh}$ until about 300 reduced time units. At this point, the encapsulation of solute slows down at both values of $\varepsilon_{hh}$ until the peak equilibrium value is reached (which, at the lower value of $\varepsilon_{hh}$, does not occur within the time frame represented in this graph). By contrast, the rate of uptake in the pre-assembled micelle case is significantly faster at $\varepsilon_{hh} = 1.2$ than it is at $\varepsilon_{hh} = 1.5$. Furthermore, both reach their equilibrium encapsulation efficiency at about 700 reduced time units.

These kinetic phenomena can be explained by the presence of an energy barrier to solute uptake in micelles that is stronger in systems where micelles form more easily. Solute uptake into a pre-assembled micelle at $\varepsilon_{hh} = 1.2$ is faster than at $\varepsilon_{hh} = 1.5$ because micelles form less easily at lower $\varepsilon_{hh}$, leading to a weaker energy barrier to entry into the empty micelle at the lower $\varepsilon_{hh}$. Meanwhile, in the co-association case, the energy barrier to uptake does not exist initially because the micelles do not exist initially. Therefore, the initial rate of uptake is similar at both values of $\varepsilon_{hh}$, and the rate of uptake changes only as micelles form with already-encapsulated solute inside.

We can gain useful information from looking at the density profiles of the solute-encapsulating micelles. A density profile is a plot of the concentration of a particular type of sphere (head, tail, solute, or solvent) as a function of the sphere’s distance from the aggregate’s center of mass. Figure 3.8 shows the density profiles of the micelles at
packing fractions $\eta = 0.15, 0.3, \text{ and } 0.4$ when at $\varepsilon_{hd} = 1.2, \varepsilon_{hh} = 1.0, \text{ and } \phi = 0.174$. The solute density profile contains a sharp peak near the center of the micelle, demonstrating the existence of a tightly packed core of solute spheres. The head profile contains a broader, lower peak that reaches its maximum outside the drug peak; this means that the tightly packed core of solute is surrounded by a less-tightly packed layer of copolymer. The tail profile contains a low peak well beyond the head peak. As can be seen from Figure 3.8, as we increase the packing fraction there is little change in the profiles; the peak heights and location do not change much, and the radius of the micelle – determined by the crossover point between head and tail – remains the same. This is surprising because as the system compresses, one would expect the size of the micelles to decrease and the density of particles within the micelles to increase. This suggests that although the system is being compressed, it is compressed in a manner that pushes solvent spheres closer to one another and that such compression does not affect the micelles much. This could explain why encapsulation efficiency appears to not be affected by packing fraction, as well as why packing fraction does not change the phase behavior of the system at high $\varepsilon_{hd}$ as determined in our previous paper.

We also looked at how the density profiles of micelles change as $\varepsilon_{hd}$ increases – the density profiles at $\varepsilon_{hd} = 1.1, 1.3, 1.4, \text{ and } 1.5$ when $\varepsilon_{hh} = 1.0, \eta = 0.3, \text{ and } \phi = 0.174$ are shown in Figure 3.9. As we can see, at the lower values of $\varepsilon_{hd}$ (1.1-1.3), the profile suggests the existence of a well-defined core of solute surrounded by a well-defined layer of block copolymer head. However, as $\varepsilon_{hd}$ increases, the head peak broadens to develop a more pronounced inner layer and the solute broadens to develop a second outer layer.
This shows that solute is distributed more evenly throughout the micelles as $\epsilon_{\text{hd}}$ increases, and that block copolymer head blocks are beginning to unfold themselves towards the center of the micelle.

Figure 3.10 shows the density profiles of the micelles at $\epsilon_{\text{hh}} = 1.2, 1.3, 1.4, \text{ and } 1.5$ when $\epsilon_{\text{hd}} = 1.2, \eta = 0$ and $\phi = 0.174$. The first interesting thing to notice is that the micelles appear to get smaller in size as $\epsilon_{\text{hh}}$ increases; that is, the point at which the head and tail curves cross moves closer to the center of mass of the micelle. The second is that the head and solute peaks exchange places over the range of values of $\epsilon_{\text{hh}}$ investigated. As $\epsilon_{\text{hh}}$ increases, the head peak becomes larger and moves towards the center while the solute peak shrinks and moves towards the outside. At the lowest value of $\epsilon_{\text{hh}}, 0.8$, there is a core of solute surrounded by a layer of head spheres; at $\epsilon_{\text{hh}} = 1.5$, this has been reversed, so that there is a core of head spheres surrounded by a layer of solute spheres. The changes in the internal structure of the micelle-solute aggregates as $\epsilon_{\text{hh}}$ increases demonstrate the stronger energy barrier to solute uptake that exists at higher values of $\epsilon_{\text{hh}}$.

The fact that the micelles get smaller as $\epsilon_{\text{hh}}$ increases, combined with the fact that the head peak grows in amplitude as $\epsilon_{\text{hh}}$ increases, shows that the more hydrophobic copolymer head blocks are forming a tighter structure that is more difficult for solute to penetrate.

### 3.4 Conclusions

In this study we used computer simulation to investigate the effect of packing fraction, polymer volume fraction, head-head interaction strength, and head-solute
interaction strength on the solute encapsulation behavior of a copolymer-solute-solvent system. Increasing the packing fraction of the system mildly decreased its encapsulation efficiency, while increasing the polymer volume fraction increased the system’s encapsulation efficiency until a maximum encapsulation efficiency was reached. The head-solute interaction strength does not have a substantial effect on the encapsulation efficiency of the system but the head-head interaction strength does. Increasing the head-head interaction strength decreases the encapsulation efficiency of the system significantly, and it does so by forcing drugs to settle on the surface of the micelle instead of becoming incorporated into the micelle core.

We also compared the encapsulation efficiency and kinetics of the system for two different nanoparticle assembly methods; one in which empty micelles were pre-assembled and then loaded with solute, and one in which solute and co-polymer co-associate. We found no difference in the equilibrium encapsulation efficiencies for the two assembly methods; however, the solute uptake kinetics of the two assembly methods are significantly different, especially at higher values of the head-head interaction strength. Furthermore, the initial speed of uptake into pre-assembled micelles is strongly dependent on $\varepsilon_{hh}$, while the initial speed of solubilization as copolymer and solute co-associate changes little with $\varepsilon_{hh}$.

We also described how the internal structure of the micelles as measured by the density profile changes as packing fraction, head-head and head-drug interaction strength change. Changes in packing fraction have no effect on the internal structure of the micelles. However, changes in both head-head and head-solute interaction strength have
significant effects on the micelle structure. Increasing the head-solute interaction strength causes the solute to be distributed throughout the core of the micelle more evenly. Increasing the head-head interaction strength pushes solute out of the micelle core toward the surface of the micelle.

We explained our results in terms of a model of solubilization proposed by Prud’homme and Kumar [16]. Their model describes the formation of an energy barrier to solute uptake around a micelle that gets stronger as more solute is loaded into the micelle. Our results, especially those that show a significant amount of solute on the surface of the micelle, are consistent with the presence of this energy barrier. Furthermore, we infer that this energy barrier is stronger when the system variables are defined so that the copolymer forms micelles more easily (for example, at high packing fraction and high head-head interaction strength).

These results suggest ways that experimentalists could boost the encapsulation efficiency of their systems. Poor encapsulation efficiency can be counteracted by changing the head block used in the copolymer formulation. The poor release profile associated with drugs remaining on the nanoparticle surface - a situation often encountered in the lab [4,5] - is likely caused by a copolymer head group that is too hydrophobic. Our research suggests that experimentalists could decrease drug surface binding and increase drug encapsulation by using a copolymer head block that is less hydrophobic than the one that causes drugs to get stuck on the micelle surface. This does not eliminate the process of trial and error in finding the right copolymer completely;
however, this research does provide a direction in which experimentalists may direct their attention in an effort to make the trial and error process more efficient.

3.5 Acknowledgements

This work was supported by the National Institutes of Health, USA under grant EB006006. We would like to thank the Graduate Assistance in Areas of National Need (GAANN) Fellowship in Biotechnology for a portion of the funding for this research. We would also like to thank Dr. Steven Smith, Dr. Andrew Schultz, and Dr. Zhengmin Li for their previous work in developing the simulation code used for this paper. Finally, we would like to thank Dr. Robert Prud’homme for conversations that helped shape this work.

3.6 References


3.7 List of Tables

Table 3.1  Matrix of interaction parameters.................................................................75
3.8 List of Figures

**Figure 3.1** Percent of solute particles encapsulated at different system packing fractions. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\phi = 0.174$..............................65

**Figure 3.2** Percent of solute particles encapsulated at varying polymer volume fraction. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\eta = 0.3$...............................................................66

**Figure 3.3** Fraction of solute particles encapsulated at varying head-head interaction strength. $\varepsilon_{hd} = 1.2$, $\phi = 0.174$...............................................................67

**Figure 3.4** Number of surface-bound solutes versus packing fraction at $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, and $\phi = 0.174$...............................................................68

**Figure 3.5** Number of surface-bound solute particles at varying head-head interaction strength. $\varepsilon_{hd} = 1.2$, $\eta = 0.3$, $\phi = 0.174$...............................................................69

**Figure 3.6** Encapsulation efficiency at various values of $\varepsilon_{hh}$ for two different means of nanoparticle assembly. $\varepsilon_{hd} = 1.2$, $\eta = 0.3$, $\phi = 0.174$...............................................................70

**Figure 3.7** Amount of solute encapsulated versus time for two different methods of nanoparticle assembly (pre-assembled micelles and co-association) at two different values of $\varepsilon_{hh}$. Blue: pre-assembly, $\varepsilon_{hh} = 1.2$. Red: co-association, $\varepsilon_{hh} = 1.2$. Green: pre-assembly, $\varepsilon_{hh} = 1.5$. Purple: co-association, $\varepsilon_{hh} = 1.5$, $\varepsilon_{hd} = 1.2$, $\eta = 0.3$ and $\phi = 0.174$...............................................................71

**Figure 3.8** Density profiles for systems at a) $\eta=0.15$, b) $\eta=0.3$, and c) $\eta=0.4$. Solute is in green, head is in black, and tail is in blue. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\phi = 0.174$........72

63
Figure 3.9  Density profiles for systems at a) $\varepsilon_{hd} = 1.1$, b) $\varepsilon_{hd} = 1.3$, c) $\varepsilon_{hd} = 1.4$, and d) $\varepsilon_{bd} = 1.5$. $\varepsilon_{h\eta} = 1.2$, $\eta = 0.3$, $\phi = 0.174$........................73

Figure 3.10  Density profiles for systems at a) $\varepsilon_{hh} = 1.2$; b) $\varepsilon_{hh} = 1.3$; c) $\varepsilon_{hh} = 1.4$; and d) $\varepsilon_{hh} = 1.5$. $\varepsilon_{hd} = 1.2$, $\eta = 0.1$, $\phi = 0.174$........................74
Figure 3.1. Percent of solute particles encapsulated at different system packing fractions. $\varepsilon_{\text{inh}} = 1.0$, $\varepsilon_{\text{hdc}} = 1.2$, $\phi = 0.174$. 
Figure 3.2. Percent of solute particles encapsulated at varying polymer volume fraction. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\eta = 0.3$. 
Figure 3.3. Fraction of solute particles encapsulated at varying head-head interaction strength. $\varepsilon_{hh} = 1.2$, $\phi = 0.174$. 
Figure 3.4. Number of surface-bound solutes versus packing fraction at $\varepsilon_{\text{hb}} = 1.0$, $\varepsilon_{\text{hd}} = 1.2$, and $\phi = 0.174$. 
Figure 3.5. Number of surface-bound solute particles at varying head-head interaction strength. \( \epsilon_{hh} = 1.2, \eta = 0.3, \phi = 0.174. \)
Figure 3.6. Encapsulation efficiency at various values of $\varepsilon_{hh}$ for two different means of nanoparticle assembly. $\varepsilon_{hd} = 1.2$, $\eta = 0.3$, $\phi = 0.174$. 
Figure 3.7. Amount of solute encapsulated versus time for two different methods of nanoparticle assembly (pre-assembled micelles and co-association) at two different values of $\varepsilon_{hh}$. Blue: pre-assembly, $\varepsilon_{hh} = 1.2$. Red: co-association, $\varepsilon_{hh} = 1.2$. Green: pre-assembly, $\varepsilon_{hh} = 1.5$. Purple: co-association, $\varepsilon_{hh} = 1.5$. $\varepsilon_{hd} = 1.2$, $\eta = 0.3$ and $\phi = 0.174$. 
Figure 3.8. Density profiles for systems at a) $\eta=0.15$, b) $\eta=0.3$, and c) $\eta=0.4$. Solute is in green, head is in black, and tail is in blue. $\varepsilon_{hh} = 1.0$, $\varepsilon_{hd} = 1.2$, $\phi = 0.174$. 
Figure 3.9. Density profiles for systems at a) $\varepsilon_{hd} = 1.1$, b) $\varepsilon_{hd} = 1.3$, c) $\varepsilon_{hd} = 1.4$, and d) $\varepsilon_{hd} = 1.5$. $\varepsilon_{\eta\eta} = 1.2$, $\eta = 0.3$, $\phi = 0.174$
Figure 3.10. Density profiles for systems at a) $\epsilon_{hh} = 1.2$; b) $\epsilon_{hh} = 1.3$; c) $\epsilon_{hh} = 1.4$; and d) $\epsilon_{hh} = 1.5$. $\epsilon_{hd} = 1.2$, $\eta = 0.1$, $\phi = 0.174$. 
Table 3.1. Matrix of interaction parameters.

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<th>Solute</th>
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</tr>
</tbody>
</table>
CHAPTER 4

Future Work

Our research focused on the computer simulation of a generic copolymer/solvent/solute system as a model for the encapsulation of drug particles within block copolymer nanoparticles. We have produced results outlining the basic phase behavior of the system as well as the overall encapsulation efficiency of the system. We have compared two different nanoparticle assembly methods and found no difference between their ultimate encapsulation efficiency, although at high head-head interaction strength the coassociating case encapsulates solute faster than uptake into previously-formed micelles. We have investigated the internal structure of the micelle/solute complex.

There are several opportunities for future work that builds upon the results of this thesis, including both simulation and experimental studies. These opportunities include: (1) variation of the size of the solute particle to include cases where one large colloid/macromolecule is being solubilized by copolymer chains; (2) use of atomistic simulation or experimental techniques to determine the values of the interaction parameters \( \varepsilon \) for several drug-copolymer pairs; (3) simulation of the solubilization of solvent-phobic solute with other polymer chain types, including triblock copolymers, dendrimers, and cross-linked polymers; and (4) simulation of the fate of drug-carrying
nanoparticles in the bloodstream, with a focus on the stability of the particles and release mechanisms for the drug. We elaborate on these ideas below.

4.1 Varying the size of the solute particle

In our model, the solute spheres and the spheres that compose the copolymer are the same size and same mass. Drugs that are used to fight cancer, however, have widely varying sizes. Small molecules like paclitaxel [1] and doxorubicin [2] are commonly used for chemotherapy; however, alternative cancer therapeutics often utilize larger payloads such as genes [3] and proteins [4] as well as complexed gold nanoparticles [5]. Our model could be adapted to simulate the encapsulation of these larger agents by modeling the drug as a large sphere with greater mass and investigating the adhesion of the block copolymer solubilizants to the surface of the sphere. Experimental work has been done on the adhesion of surfactants onto silica particles; researchers have found that the adhesion of copolymers onto the particle surface begins to occur at the critical micellization concentration of the copolymer in bulk solution [6-7]. Simulations could determine whether or not this phenomenon is universal or if it is limited to this particular copolymer-particle pair. Furthermore, simulations could determine whether the ease of micellization of the copolymer in solution impedes the adhesion of copolymer onto the particle surface in the same manner that ease of micellization of the copolymer impedes solute uptake into the micelle in this thesis. Work in this area will be the subject of a forthcoming paper by Woodhead and Hall.
4.2 Determining values of $\varepsilon$ for copolymer-drug pairs

Our model tests a range of values of the interaction parameter $\varepsilon$ and determines the effect of greater attraction between copolymer head spheres and solute as well as between copolymer head spheres and other copolymer head spheres. These values of $\varepsilon$, however, are relative and do not represent any specific copolymer/solute pair. Having the ability to assign $\varepsilon$ values to actual copolymer-drug systems could allow our model to serve as a predictive tool for cancer drug encapsulation. Numerous methods currently exist that could be used to determine the $\varepsilon$ values of commonly used copolymer-drug pairs. Atomistic simulation can be used to gauge the strength of the hydrophobic interaction [8]. Should this be impractical, interaction parameters can be estimated using equations of state [9] or functional group contribution [10]. These can be compared to experimental values found by inverse gas chromatography [11]. Once this table has been generated, the theoretical methods could also be used to predict other novel copolymers that could better encapsulate each drug, and these copolymers could be synthesized and tested in the lab.

4.3 Simulation of other polymer drug delivery vehicles

Our simulation concentrates on the ability of block copolymers to encapsulate drugs. However, other polymer-based experimental drug delivery vehicles are also being used for this purpose. Researchers have used liposomes [12], triblock copolymers such as
Pluronics [13], cross-linked copolymers [14], and dendrimers [15] to encapsulate various anti-cancer drugs. Simulation of drug-carrying liposomes is currently the subject of research by Curtis and Hall; adaptations to our model could easily be done to simulate triblock copolymers. Our current simulation code could also be modified to simulate branched polymers such as dendrimers and cross-linked polymers. Simulations could be performed that determine the encapsulation efficiency and phase behavior of these nanoparticle-forming compound types at various values of the interaction parameters. It would be interesting to see if there are any parallels between drug encapsulation within these other types of molecules and the results reported in this thesis. Furthermore, dendrimers have been synthesized that have pH-dependent release mechanisms [15]; this could be simulated as well in order to understand the kinetics of drug release by this mechanism.

4.4 Simulation of drug-carrying nanoparticles in the body

In order for drug-carrying block copolymer nanoparticles to be viable as anti-cancer therapeutics, they must not only encapsulate drugs well, but also be stable in the bloodstream and release their drug payload into the tumor in a predictable fashion. Our simulation covers only the first portion of this process. Simulations would be performed on dilute solutions of nanoparticles in an aqueous environment in order to see if they dissolve when the copolymer concentration decreases below the critical micellization concentration. Starting with the final configuration of the simulations conducted in this
thesis, we would eliminate several copolymers and solute spheres that are part of micelles and replace them with solvent spheres. We would then allow the system to continue to run, measuring the rate of drug encapsulation and release and micelle decay over time. The rate of drug release would be measured for several different values of the system packing fraction, head-head interaction strength, and head-solute interaction strength. If certain parameters that lead to good drug encapsulation also form unstable nanoparticles, this could serve as a further restriction on the parameters that lead to viable drug-carrying nanoparticles.

We could also simulate release mechanisms that allow the drug to be released in the presence of the tumor. For example, researchers have developed pH-sensitive copolymer micelles that can release the drug at the lower pH found near tumor cells [16]. This could be simulated by treating the low-pH solvent as a reactive solvent that can change the properties of tail or head spheres after a core collision. We could vary the reaction kinetics by changing the probability of a reaction after a core collision between solvent and copolymer, and we could vary the pH by changing the number of solvent particles that can react with the copolymer.

### 4.5 References


