Polymer Reservoirs to Solubilize Hydrophobic Drugs

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Dedication

Dedicated to mom, dad, and Wenjia.
Abstract

Research and development of new drug delivery formulations for hydrophobic drugs hold great promise for patients worldwide in the ever-growing pharmaceutical industry. A large portion of the drugs, both in the current market and the development pipeline, suffer from low aqueous solubility, therefore limiting their efficacy for oral administration. One effective way to resolve this problem is the use of an amorphous solid dispersion (ASD), a blend of drug and polymer. An ideal polymer candidate can kinetically stabilize the dispersed drug in its amorphous form in the solid state, while enhancing drug solubility and dissolution in the solution state. Despite recent advances in polymer development for oral drug delivery, the structure-property relationships and the underlying solubility enhancement mechanisms are not fully understood for ASDs. The goals of this dissertation are to develop effective polymers for oral drug delivery, and more importantly, to elucidate the mechanism(s) of drug solubility and dissolution enhancement by using well-defined polymer platforms. Specifically, three model systems were designed and synthesized, including blends of a commercially available polymer and self-assembled micelles in Chapter 3, micelle-forming statistical copolymers and diblock polymers in Chapter 4, and chemically crosslinked polymer nanogels in Chapter 5. It was observed universally in all these three systems that hydrophobic drugs can be sequestered in the slightly hydrophobic polymer reservoirs, and that the drug-polymer partitioning is stronger when the polymer chains are more crowded. The partitioning inhibits drug nucleation and crystal growth in aqueous solution, resulting in enhanced drug solubility. This mechanism is supported by a battery of state-of-the-art characterization experiments, including light scattering, nuclear Overhauser effect and diffusion ordered spectroscopy, cryogenic transmission electron microscopy, small-angle X-ray scattering, and in vitro dissolution tests. Potential applications of the discovered mechanism and the characterization experiments to other drug/polymer systems are discussed as future directions.
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1. Introduction

1.1 Opportunity and Challenge of Polymers in Oral Drug Delivery

Research and innovation of new pharmaceuticals has a profound impact on human life expectancy and quality. In the past decade, the pharmaceutical industry faced an enormous challenge because of its business model. The global pharmaceutical market has recently been reported to exceed $1.1 trillion in annual sales. However, there is a constant pressure for pharmaceutical companies to reduce their product prices, while the cost of drug development has been increasing. In fact, approximately only one out of every 10,000 drug candidates can ultimately make it to the market. Partially due to such high attrition rate, the average time and cost to bring a new active pharmaceutical ingredient (API) through clinical trial and regulatory approval to the market are at least 11 years and $800 million, respectively. More than 80% of drug candidates in the development pipeline suffer low aqueous solubility, and this will cause poor oral bioavailability. The majority (i.e., 60-70%) of the APIs in the pipeline that suffer low aqueous solubility are classified as Biopharmaceutics Classification System (BCS) Class II compounds, which have high permeability through the human intestinal membrane but low solubility. Nevertheless oral drug delivery remains the predominant route of administration for a variety of reasons, including ease of ingestion, patient compliance, and low manufacturing cost. Thus, one of the major challenges for oral drug formulation is to resolve the low aqueous solubility issue for these hydrophobic drug molecules.

Polymers, large molecules composed of many covalently bonded repeat units, play an important role in oral drug delivery. They offer a multi-dimensional platform for researchers in formulation design to resolve the solubility issue and ultimately improve oral bioavailability of hydrophobic drugs. Physical and chemical properties of a polymer
can be tuned by changing its molecular weight, chain architecture, chemical structure of the repeat units, etc. All these design parameters can dictate the performance of a drug/polymer formulation. For example, it has been shown that molecular weight of a polymer can affect drug-polymer partitioning strength,\textsuperscript{11} drug \textit{in vitro} release rate,\textsuperscript{12} and solubility enhancement.\textsuperscript{13} Polymer chain architecture and chemical structure, on the other hand, can dictate behavior in aqueous solution. Polymer assemblies, such as core-shell micelles, are extensively studied and often used as nanocarriers for oral drug delivery, as discussed in recent review papers.\textsuperscript{14,15} Therefore, finding an optimum combination of polymer design parameters in this multi-dimensional platform can improve drug efficacy, and this can be both challenging and rewarding.

How can we turn the challenges into opportunities? In fact, there are already a variety of polymers that have been designed, approved by the U.S. Food and Drug Administration (FDA), and commercialized as oral therapeutic products in the past few decades, as discussed in a recent review by Ting et al.\textsuperscript{16} The most widely-used and commonly recognized polymers for oral drug delivery are polyvinylpyrrolidone (PVP),\textsuperscript{17,18} poloxamers (also known as Pluronics),\textsuperscript{19,20} poly(acrylic acid),\textsuperscript{21} and hydroxypropyl methylcellulose (HPMC) and its derivatives.\textsuperscript{22–24} The chemical structures of these polymers differ significantly, therefore they can be used to target a wide range of hydrophobic drugs with different chemical and physical properties. Furthermore, physical blends and/or copolymers can also be used as complementary methods to solubilize hydrophobic drugs, if one single polymer does not work well. For example, Marks et al. have investigated the miscibility of a few polymer blends of cellulose-based polymers (e.g., HPMC) and synthetic polymers (e.g., PVP) for oral drug delivery.\textsuperscript{25} For the poorly water-soluble antiretroviral drug ritonavir, Ilevbare et al. showed that the blending two polymers with different properties can result in improved drug crystal growth inhibition, compared to each individual polymer.\textsuperscript{26} In addition, Barreiro-Iglesias et al. have grafted
PVP onto poloxamers and compressed these graft polymers with three different drugs (theophylline, hydrochlorothiazide, and nitrofurantoin) to produce tablets as oral dosage formulations for enhanced drug release properties.\textsuperscript{27}

All the above-mentioned studies have demonstrated great potential opportunities in polymer design. As shown in a recent review by Williams et al., significant advances have been made in the past decade to identify what type of polymer works well for a specific model drug.\textsuperscript{28} In the review, a variety of common strategies to improve the low drug aqueous solubility are discussed, including the use of cosolvents, salts, surfactants, cyclodextrins, particle size reduction, polymorphs, lipid-based systems, cocrystals, and amorphous solid dispersions (ASDs).\textsuperscript{28} In this thesis, we will focus on the use of ASDs, which are mixtures of API and polymer. What has been learned so far about the ASDs will be discussed in Section 1.2, including physical stability in the solid state and dissolution enhancement in the solution state. Despite the recent advances, the underlying mechanism of the drug dissolution enhancement by using an ASD, especially the role of the polymer, is not fully understood. For hydrophobic drugs, enhancing the aqueous solubility means that the system is in a nonequilibrium state (i.e., supersaturation), and therefore one of the major challenges in this field is to better understand how these nonequilibrium systems are achieved and stabilized. In Section 1.3, the commonly observed drug dissolution profiles when using ASDs and their corresponding mechanisms will be discussed. In addition, micelles and nanogels will be discussed as examples to demonstrate typical loading and release mechanisms when using polymer nanostructures in ASDs. In Section 1.4, the current state-of-the-art characterization techniques for ASD systems will be briefly reviewed. A few novel characterization experiments developed in the past 5 years will be discussed and these experiments can help to elucidate the underlying mechanism of drug dissolution enhancement. Section 1.5 presents the goal and outline of this dissertation.
1.2 Strategy to Solubilize Hydrophobic Drugs – Amorphous Solid Dispersion

The use of ASDs, first reported by Sekiguchi and Obi in 1961,\textsuperscript{29} is often considered as one of the most effective strategies to enhance the efficacy of poorly water-soluble drugs. Despite the research and development in the past few decades, the drug dissolution enhancement and supersaturation stabilization mechanisms by using the ASDs are still not fully understood.\textsuperscript{30} APIs generally show higher solubility in their amorphous form than in crystalline form (up to ~50 fold increase for some drugs\textsuperscript{31}), because the amorphous form has a higher chemical potential.\textsuperscript{32} Unfortunately, this higher chemical potential also means that amorphous APIs have a strong tendency to nucleate and crystallize into the more thermodynamically stable state, the crystalline form. Polymers, as an inactive ingredient in ASDs, play an important role to stabilize APIs in the desired thermodynamically unstable amorphous form, by inhibiting crystal nucleation and/or growth. In fact, the physical and chemical interactions between polymers and APIs can dictate the performance of a given ASD both in solid and solution states (e.g., shelf-life and solubility). Therefore, there are many opportunities to explore in order to improve the ASD performance, and ultimately, to elucidate the underlying mechanism(s).

The two most commonly used processing methods to produce amorphous solid dispersions are hot melt extrusion (HME) and spray drying. A HME process incorporates the API and polymers into a kinetically miscible blend by mixing them at elevated temperatures in a molten state. HME offers some advantages, including fewer processing steps, continuous operation, and absence of solvents, over other traditional pharmaceutical processing techniques.\textsuperscript{33} However, a major concern associated with this method is the potential thermal degradation of the API. In contrast, a spray drying process converts a liquid feed that dissolves both API and polymers into spray-dried dispersions (SDDs) by rapid evaporation of the solvent. The liquid stream is dispersed into an enormous number of tiny droplets and sprayed in the processing gas (e.g., hot
The rate of solvent evaporation is presumably much faster than the rate of solute diffusion and crystallization during this process, which kinetically traps the drug into an amorphous state. Spray drying is preferred when dealing with high glass transition temperature ($T_g$) polymers, due to reduced concern about the API degradation.

No matter which processing method is chosen, physical stability is an extremely important criterion for amorphous solid dispersion formulations due to the thermodynamic drive to convert the drugs into the more stable crystalline form. ASDs are typically non-equilibrium systems due to the kinetic aspects of processing procedure (e.g., rapid solvent evaporation during the spray drying process). Depending on the kinetic conditions of the processing procedure, there are three possible final states of the solid dispersions: (1) homogenous dispersion of amorphous drug molecules in the polymer matrix, (2) phase-separation into the polymer-rich and/or drug-rich domains, and (3) crystallized drugs in the polymer matrix. The existence of any drug crystals in the solid state can induce a rapid crystallization of the remaining drug molecules upon dissolution in aqueous solution, and therefore, results in poor oral bioavailability. In addition, the formation of separated drug-rich and polymer-rich phases in the solid state will increase the possibility of drug nucleation and crystal growth. Rumondor et al. posited that drugs can crystallize either immediately from a one-phase ternary system, or after the formation of a drug-rich region upon amorphous-amorphous phase separation, when the ASDs are exposed to moisture. Such amorphous-amorphous phase separation will most likely induce drug crystallization, because of the lack of polymers in the drug-rich phase. Thus, it is important to understand the molecular arrangement of the drugs and polymers in solid dispersions. A variety of experimental techniques can be used to investigate the miscibility of the prepared ASDs to ensure good physical stability (e.g., shelf life), and these possible characterization techniques will be discussed in Section 1.4.
Ultimately, the efficacy of an oral dosage formulation is evaluated after its dissolution in aqueous solution. In solution, the enhanced drug solubility comes from the free energy difference between the amorphous and crystalline forms, and such difference naturally drives drug crystallization and subsequent precipitation in solution. Upon dissolving the ASDs in aqueous media, the degree of supersaturation ($S$) is defined as the ratio of the amorphous drug concentration ($c$) to the crystalline drug solubility ($c_{\text{cryst}}$), as shown in the following equation:\textsuperscript{39,40}

$$S = \frac{c}{c_{\text{cryst}}} = \exp \left( \frac{\Delta G}{RT} \right)$$ \hspace{1cm} (1.1)

where $\Delta G$ is the free energy difference between the amorphous and crystalline drugs, $R$ is the universal gas constant, and $T$ is the absolute temperature. The drug dissolution rate, on the other hand, is highly dependent on the surface area as shown by the Noyes and Whitney equation:\textsuperscript{41}

$$\frac{dc}{dt} = \frac{DA(c_s - c)}{h}$$ \hspace{1cm} (1.2)

where the drug dissolution rate $dc/dt$ is governed by diffusion coefficient of the drug ($D$), surface area of the drug in contact with the dissolution media ($A$), thickness of the diffusion layer ($h$), drug concentration at the surface of the dissolving solid ($c_s$), and concentration of solute in the bulk solution ($c$). ASDs generally have high surface area and porosity,\textsuperscript{42,43} and therefore, can facilitate rapid drug dissolution when exposed to aqueous solution. In the absence of any polymer excipient, a higher drug dissolution rate will lead to a higher attainable solubility but a faster decline in the drug supersaturation.
level, based on both experimental and modeling work. Polymers can substantially prolong the drug supersaturation period by inhibiting drug nucleation and/or crystal growth, and therefore, increase oral bioavailability. Thus, it is important to understand the drug dissolution enhancement mechanism when using ASDs. The correlation between the dissolution profiles and the corresponding enhancement mechanism will be discussed in the following section.

1.3 Drug Dissolution Enhancement Mechanism – Role of Polymer

In this section, examples of commonly observed drug dissolution profiles of ASDs and their corresponding mechanisms will be discussed. In addition, typical drug loading and release mechanisms associated with two types of nanoparticles, micelles and nanogels, will be briefly reviewed. For oral delivery of ASDs, solubility and permeability are the two most important parameters that dictate the ultimate drug bioavailability. This dissertation focuses on the solubility issue of hydrophobic drugs, and all the model drugs investigated in this work are BCS Class II compounds, which have low solubility but high permeability.

There are tens of thousands of drug candidates in the discovery and development pipeline, and therefore it is inefficient to design and optimize a specialized polymer for each candidate without a basic understanding of the drug dissolution enhancement mechanism(s). Unfortunately, no single mechanism can be universally applied to all drugs, polymers, and their combinations. Nevertheless, some commonly accepted mechanisms can be used to predict the solution-state behaviors of a set of drugs with similar physical properties (e.g., hydrophobicity and rate of crystallization), and they will be valuable for rational design of the polymers. The role of the polymer in an ASD will be the focus of discussion in this section. Upon dissolution of ASDs in aqueous solution,
different dissolution profiles can occur as shown in Figure 1.1, depending on the chemical and physical properties of the polymers. The supernatant drug concentration measured during a standard microcentrifuge dissolution assay represents a combination of both molecularly dissolved drugs and drugs that partition to polymers in the form of drug-polymer nanoparticles. Crystalline drugs will precipitate out of the solution, and therefore will not be present in the dissolution supernatant. In an ideal case as shown in Scenario (a) in Figure 1.1, drugs are rapidly dissolved and dispersed in aqueous solution at the highest possible target concentration (i.e., typically much higher than the crystalline solubility), and the drug supersaturation can be maintained throughout the residence time of the drug in the human gastrointestinal (GI) tract. This desired scenario has been achieved by using three different polymer systems (i.e, blend, micelles, and nanogels) in Chapters 3-5 of this dissertation. In all three cases, the model drugs partition strongly to the polymers in the form of drug-polymer nanoparticles, resulting in an ideal solubility enhancement and maintenance by preventing drug crystallization throughout the dissolution period.
Guzman and coworkers have introduced the concept of combining a rapidly dissolving and supersaturating “spring” with a crystallization inhibiting “parachute” to achieve and maintain high drug availability, as shown in Scenario (b) in Figure 1.1. Amorphous drug has a thermodynamic drive to solubilize at a concentration higher than its equilibrium value. In the absence of polymers, these supersaturated drug molecules will rapidly nucleate and grow into crystals, resulting in a rapid decrease of drug concentration due to precipitation. Therefore, an ideal polymer should not only rapidly dissolve the drugs (i.e., “spring” effect), but also act as a “parachute” to maintain the drugs in a supersaturated state by inhibiting drug nucleation and/or crystal growth. If the highest possible supersaturation is maintained by the polymers throughout the absorption period, Scenario (b) essentially becomes Scenario (a). The most commonly invoked mechanism in the literature to explain the role of polymer in the drug nucleation
and crystal growth retardation processes is the existence of strong drug-polymer interactions. It has been demonstrated that the effectiveness of crystal growth inhibition can be directly correlated with the strength of hydrogen bonding between the drug and polymer, suggesting that drug-polymer intermolecular interactions such as hydrogen bonding and hydrophobic interactions can play an important role in crystal growth inhibition.\(^{47-49}\) For example, a variety of polymers (e.g., poly(acrylic acid), poly(vinylpyrrolidone), and hydroxypropyl methylcellulose acetate succinate) have been shown to inhibit drug crystal growth by adsorbing to the growing crystal surface via hydrogen bonding.\(^{47-51}\) Such favorable drug-polymer interactions can delay or prevent drug crystallization by disruption of the critical drug-drug interactions.

There are certainly ASD systems that do not follow the “spring and parachute” predicted dissolution profile (i.e., Scenario (b) in Figure 1.1). As shown in Scenario (c) in Figure 1.1, it has been observed in multiple studies that an intermediate drug supersaturation (i.e., lower than the target one) can be achieved initially and maintained throughout the entire dissolution period (i.e., typically 4-6 hours).\(^{11,13,52}\) A few hypotheses are: (1) the drugs dissolve initially but crystallization occurs very rapidly so that the standard dissolution tests could not capture the high drug concentration at early times, (2) the amount of polymer is only able to stabilize a certain fraction of the drug molecules at a supersaturated concentration while the remaining drugs crystallize and precipitate, (3) drugs remain amorphous in the form of some large drug/polymer aggregates, some of which precipitate due to high hydrophobicity. In order to further improve the drug solubility enhancement of ASDs exhibiting dissolution profiles as shown in Scenario (c) of Figure 1.1, it is important to understand which form do these supersaturated drugs exist in solution, and where are the remaining drugs. Last, some ASDs can exhibit a slowly increasing drug concentration upon dissolution, as shown in Scenario (d) in Figure 1.1. For example, Ting el al. and Ricarte el al. have both observed
slowly increasing dissolution profiles when using probucol as the model drug, with two different polymers.\textsuperscript{53,54} Ricarte el al. have shown that such slowly increasing dissolution profile was caused by the slow disintegration of the ASDs in solution over time, when using ASDs of probucol and HPMCAS.\textsuperscript{54} Such a dissolution profile was also observed in Chapter 5 with one ASD system using crosslinked polymer nanogels and the dissolution profile was directly correlated with the nanoparticle evolution. Therefore, investigation of solution-state structures of the ASDs during dissolution is the key to better understand the underlying mechanisms.

In 2008, Friesen et al. proposed that drug/polymer nanostructures will form upon dissolution of the ASDs in aqueous solution, and rapid exchange can exist between free drugs and drugs in the drug/polymer nanostructures.\textsuperscript{24} When the freely dissolved drugs permeate through the intestinal membrane, the drugs stored in these nanoparticles can rapidly replenish the adsorbed ones and maintain the high drug supersaturation level.\textsuperscript{24} The formation and stability of these presumably drug-rich nanoparticles might therefore be highly correlated with the mechanism(s) of the drug solubility and dissolution enhancement. Recent studies in our groups have shown that there is rapid exchange between molecularly dissolved drugs and drugs that partition to the polymer micelles, based on the 2D \textsuperscript{1}H diffusion ordered spectroscopy NMR results (details are shown in Chapters 3-4).\textsuperscript{11,55} Polymers can serve as reservoirs to facilitate formation of the drug/polymer nanostructures in solution, which are directly correlated with the resulting dissolution profiles of the ASDs (Figure 1.1).\textsuperscript{11,54,55} A similar concept was discussed comprehensively in a recent review by Taylor and Zhang.\textsuperscript{56} Liquid–liquid phase separation can occur, resulting an equilibrium between a supersaturated aqueous solution and drug-rich nanodroplets, when the achieved drug supersaturation concentration is higher than its amorphous solubility for slowly crystallizing drugs.\textsuperscript{56} In this dissertation, nanostructures such as micelles (Chapters 3-4) and crosslinked nanogels (Chapter 5) were
also used as reservoirs to stabilize the high drug supersaturation by preventing crystallization. Thus, the commonly accepted drug solubility enhancement mechanisms associated with the nanostructures will be briefly reviewed, by using micelles and nanogels as two examples in the following discussion.

In fact, there has been a growing interest recently in both industry and academia to use polymers that form nanostructures in drug delivery applications, such as micelles and crosslinked hydrogels. The loading and release of hydrophobic drugs are the two key processes to achieve the desired drug dissolution profile (i.e., Scenario (a) in Figure 1.1). In aqueous solution, amphiphilic block polymers can assemble into a micellar structure, consisting of a hydrophobic core that is stabilized by a hydrophilic shell. Because of the hydrophobicity, the core is expected to be an ideal pocket to store poorly water-soluble drugs due to favorable hydrophobic interactions. Hydrophobic drugs can often be loaded and entrapped in the micelle cores during the micelle assembly process by dialysis. The shell, on the other hand, maintains the drug-loaded core solubilized and dispersed in aqueous solution. Polyethylene oxide (PEO), also known as polyethylene glycol (PEG), is one of the most commonly used hydrophilic blocks for this purpose. For example, poly(lactide)-b-poly(ethylene glycol) (PLA-b-PEG) diblock micelles have been used to load two different model drugs, griseofulvin and itraconazole, for oral administration in two studies.

One major challenge associated with encapsulating hydrophobic drugs in a core-shell micelle is the subsequent drug release. From a thermodynamic standpoint, the formed micelle should be stable when the actual dosage concentration is above the critical micelle concentration (CMC). In practice, these micelles can be diluted to concentrations below the CMC after introduced into the gastrointestinal (GI) tract. As a result, the thermodynamically unstable micelles can dissociate into free chains, potentially offering an opportunity to unload the encapsulated drugs. However, programming micelle
dissociation and drug unloading in a controlled manner can be extremely difficult. For example, the micelle core can be kinetically trapped when using a hydrophobic block with high glass transition temperature \( (T_g) \).\(^6\) To address the controlled release problem, a variety of stimuli-responsive polymers have been widely used to trigger drug release by changes in pH and temperature.\(^6\)–\(^8\) Thermo-responsive polymers with lower critical solution temperatures (LCSTs) in aqueous solution, such as poly(N-isopropylacrylamide) (PNIPAm), swell at low temperature but shrink in size and ultimately collapse into a polymer-rich domain when heated above the LCST. Chuang et al. have prepared block polymers of poly(N-isopropylacrylamide-\(b\)-butylmethacrylate) (PNIPAm-\(b\)-PBMA) and the model drug adriamycin was loaded in the PBMA core.\(^7\) The release of the loaded drugs was triggered by heating the system above the \( T_g \) of the PBMA core block (i.e., 20 °C).\(^7\) In this case, the drugs were released because the PNIPAm-\(b\)-PBMA micelle deformation can be achieved with a low \( T_g \) PBMA core block.\(^6\),\(^7\) In contrast, for a high \( T_g \) core block like polystyrene (PS, \( T_g = 100 \) °C), Chang et al. have showed that PNIPAm-\(b\)-PS micelles retained the structure over the temperature range from 20 to 40 °C, despite the changes of the PNIPAm corona.\(^6\)

During oral administration, pH changes significantly, from the highly acidic environment in the stomach to a relatively neutral pH in the small intestine.\(^7\) Thus, micelles with core blocks that contain ionizable weakly acidic groups can also be used to trigger drug release in the intestinal environment. For example, Jones et al. synthesized hydrophobic ethyl methacrylate/\(\text{tert}\) -butyl methacrylate and hydrophilic poly(ethylene glycol)methacrylate star polymers, and formed an ionizable micelle core with methacrylic acid function by hydrolysis of the \(\text{tert}\)-butyl methacrylate.\(^7\) The \textit{in vitro} release rate of the micelle-loaded hydrophobic model drug, progesterone, was increased from the gastric environment of pH=1.2 to the small intestinal environment of pH=7, due to the ionization of the micelle core.\(^7\)
The other nanostructure investigated in this dissertation (in Chapter 5) is crosslinked polymer nanogels. Nanogels, also known as microgels, are crosslinked polymeric particles with a tunable size from nanometers to micrometers.\textsuperscript{74} These crosslinked particles can be ideal drug delivering vehicles because of the tunable particle size and chemical functionality. Nanogels with highly hydrophilic chains can swell dramatically in aqueous solution (e.g., sometimes hydrated up to 90 wt%). Chemically crosslinked microgels/nanogels are often synthesized by emulsion polymerization, and the relevant chemistry and experimental details will be discussed in Chapter 2. Ever since the first introduction of synthetic hydrogels by Wichterle and Lim in 1960,\textsuperscript{75} these materials have been studied extensively for drug delivery applications.\textsuperscript{76}

There are various ways to load different drug molecules into the crosslinked structures. Das et al. have shown that two different model drugs, ornidazole and ciprofloxacin, can be homogenously mixed with the crosslinked poly(N-isopropylacrylamide) (PNIPAm) hydrogel and compressed into a final tablet formulation.\textsuperscript{77} Slemming-Adamsen et al. loaded the anti-cancer drug doxorubicin into a PNIPAm/gelatin nanofiber \textit{in situ} during the crosslinking process by electrospinning.\textsuperscript{78} Liu et al. were able to load the same model drug into a temperature and pH dual-stimuli responsive poly(methacrylic acid)/poly(N-isopropylacrylamide) (PMAA/PNIPAm) microgel by dissolving both the drug and microgel in aqueous solution and shaking for 48 h.\textsuperscript{79} Similar to micelles, controlled release can also be realized in the microgel/nanogel case by having stimuli-responsive function groups. For example, in the study of Liu et al., the release of doxorubicin is readily tunable with the pH-sensitive PMAA core and the temperature-sensitive PNIPAm shell.\textsuperscript{79} In fact, the underlying controlled drug release mechanisms (e.g., by changes in pH and temperature) for the crosslinked particles are often the same as for micelles. Altogether, there are tremendous opportunities for researchers to explore in the polymer field (e.g., materials science and processing
approaches) to obtain efficient stimuli-responsive nanoparticles such as micelles and nanogels for controlled release. The characterization of these nanoparticles is enabled by a variety of the experimental techniques, which can help to investigate the underlying drug solubility enhancement mechanisms. The advantages and drawbacks of each technique will be discussed in the following section in more detail.

1.4 Characterization of Amorphous Solid Dispersions

Characterization techniques for ASD physical stability will be presented first in this section, including miscibility and drug crystallinity. These are the important parameters to ensure the ASD integrity in the solid state. Ultimately, the performance of an ASD formulation needs to be evaluated in the solution state. Thus, characterization of the ASD solution behavior will be the focus of this section, with emphasis on how to elucidate the dissolution mechanisms by a combination of different characterization techniques.

Miscibility – Thermodynamic vs. Kinetic

The most commonly used technique to probe the miscibility of an ASD is differential scanning calorimetry (DSC), and a distinctive single glass transition temperature (i.e., intermediate between the $T_g$ values of pure drug and pure polymer) obtained from the DSC can indicate homogenous mixing. In contrast, the detection of multiple $T_g$s with near pure component values suggests inhomogeneity of the dispersion (e.g., phase separation). Moreover, modulated differential scanning calorimetry (MDSC) can be used to resolve peak overlapping issues that often arise during DSC characterization. In this mode, simultaneous linear and sinusoidal heating rates are imposed to allow the deconvolution of the total heat flow rate $dH/dt$ into a linear combination of the reversing
component related to the heat capacity \( C_p \) (d\( T \)/dt), and the non-reversing component \( f(T,t) \), as shown in Equation 1.3.

\[
\frac{dH}{dt} = C_p \frac{dT}{dt} + f(T,t)
\] (1.3)

The physical stability of an ASD can be predicted by its glass transition temperature. Glass stabilization, a strategy by which the dispersion is stored 40 to 50 °C below its \( T_g \), is usually used to ensure the shelf life of the ASDs.\(^{82}\) Hence, high-\( T_g \) polymers are typically better candidates for storage considerations. In most cases, the first heating curve is often used to determine the mixing state of the ASDs as prepared, to avoid measuring a potentially altered ASD due to exposure to elevated temperatures (\( T > T_g \)) during the DSC measurements.\(^{53}\)

Unfortunately, a distinctive single \( T_g \) might not be a reliable indicator of homogeneity in some cases (e.g., polymer and drug with similar \( T_g \)).\(^{83}\) Therefore, other techniques may be required to definitively determine the miscibility of the ASDs as complements to DSC. Infrared (IR) and Raman spectroscopy can be used to investigate drug-polymer interactions. For example, Taylor and Zografi have observed a progressive decrease in the peak wavenumbers and peak widths as the concentration of PVP in the solid dispersions increases in both the Raman and IR spectra, which indicates the existence of hydrogen bonding between model drug indomethacin and PVP.\(^{84}\) However, the spatial resolution of both infrared and Raman spectroscopy (i.e., typically greater than a micrometer) limits the molecular-level understanding of the drug/polymer miscibility.\(^{85,86}\) Solid-state nuclear magnetic resonance (NMR) spectroscopy has been used to investigate the mobility of small drug molecules in a polymer matrix on the molecular level.\(^{87}\) In addition, Schantz et al. have successfully used solid-state NMR to probe the molecular
interactions between a model drug paracetamol and citric acid anhydrate, in both physical mixtures and amorphous blends. Recently, Ricarte et al. have shown that electron energy-loss spectroscopy (EELS), coupled with transmission electron microscopy (TEM), is capable of revealing structure and quantifying chemical composition of solid dispersions with sub-100 nm spatial resolution. These experimental techniques can be used in tandem with each other to characterize miscibility of the ASDs.

**Drug Crystallinity**

Drug crystallinity is another important aspect in ASD characterization since the ultimate drug bioavailability in an ASD is highly dependent on the amount of drug crystals in the solid state. In some cases, simple MDSC experiments allow the quantification of the percent drug crystallinity in ASDs. For example, Ting et al. have precisely calculated the probucol crystallinity in ASDs using MDSC and found that the crystallinity content increased as the drug loading increased, from no crystallinity at 10 and 25 wt% probucol loadings to more than 20% crystallinity at 50 wt% probucol loading for ASDs using hydroxypropyl methylcellulose acetate succinate (HPMCAS). However, DSC will not work for ASDs if the polymer degrades before the drug melts during heating.

Wide angle X-ray scattering (WAXS) has been commonly used to detect the existence of crystals in solid dispersions, where the Bragg peaks corresponding to the unique crystal structure of the crystalline drug will appear on top of the broad halo from the amorphous polymer. The overall extent of crystallinity can be quantified by dividing the area of the Bragg peaks (after background subtraction) by the total area of the diffraction pattern. The lower detection limit of a laboratory-scaled WAXS is reported to be approximately 5 vol%, though some researchers believe it can be sensitive to about 1
vol\% crystallinity.\textsuperscript{91} In contrast to laboratory-scale WAXS, synchrotron-source WAXS can deliver not only a much lower limit of detection of crystalline (about 0.2 vol\%) but also provide the capability to perform \textit{in situ} crystallization experiments with an extremely high time resolution of 40 ms.\textsuperscript{92} In an aging study, Bhardwaj et al. used synchrotron WAXS to detect early stages of itraconazole crystallization after 29 days of storage, whereas the laboratory-scaled WAXS did not reveal any drug crystallization until after 59 days.\textsuperscript{93} In this study, WAXS was also used to determine the itraconazole crystallization kinetics (i.e., fraction of crystallized drug over time) at elevated temperatures in the presence or absence of polymer, by a quantitative analysis of the 2D synchrotron scattering patterns.\textsuperscript{93} Other than DSC and WAXS, solid-state NMR and TEM can also be used to assess the drug crystallinity in solid dispersions. For example, Ueda et al. have demonstrated that solid-state NMR relaxometry can enable the investigation of the nifedipine drug crystals in a polymer matrix with a domain size of 80-90 nm or less.\textsuperscript{94} Maruyoshi have used solid-state NMR to quantify two distinct crystalline phases of cimetidine in a physical mixture, down to a detection limit of 1 wt\%.\textsuperscript{95} Ricarte el al. have explored the use of TEM to detect griseofulvin crystals in a solid dispersion with HPMCAS with nanoscale spatial resolution and they have also demonstrated that TEM has superior crystal detection sensitivity (i.e., \(< 3\) vol \% based on this study) than DSC and laboratory-scaled WAXS (both have crystal detection limit \(> 5\) vol \%), as shown in Figure 1.2.\textsuperscript{96}
Figure 1.2. (a) In situ WAXS of 5 wt % griseofulvin solid dispersion before and after annealing at 140 °C. Similar to the modulated DSC results, the diffraction patterns of the sample during annealing do not show signs of crystallinity. (b) Bright-field TEM, (c) electron diffraction, and (d) dark-field TEM reveal that some particles in the post-annealed in situ WAXS sample contained griseofulvin crystals. Figure adapted from Ricarte, R. G.; Lodge, T. P.; Hillmyer, M. A. Mol. Pharm. 2015, 12, 983–990. Copyright 2015 American Chemical Society.

Solution Behavior

Solution-state studies are critical to evaluate the performance of amorphous solid dispersions since these formulations will ultimately be used in an aqueous environment. This project targets the oral delivery of hydrophobic drugs, and therefore, aqueous solubility is the most important solution-state parameter. The apparent concentration of the supersaturated drug is often quantified as a function of time by some biorelevant in vitro dissolution tests. One commonly used in vitro dissolution test is performed using a United States Pharmacopeia (USP) apparatus under sink conditions (i.e., SI > 10 in Equation 1.4), which is ideal to evaluate disintegration of the drug-polymer formulation and rate of drug release. However, discrimination among different dissolution profiles
can be difficult using sink conditions due to fast dissolution with a high volume of solvent.\textsuperscript{98} For ASDs loaded with hydrophobic drugs, drug supersaturation is often achieved upon dissolution under non-sink conditions (i.e., SI < 0.1 in Equation 1.4).\textsuperscript{99} Thus, non-sink dissolution conditions are considered to be more appropriate than conventional USP protocols, especially when crystallization and precipitation are big concerns during the dissolution process.\textsuperscript{97,100} The micro-centrifuge dissolution assay is a widely used protocol, to simulate conditions in the intestinal lumen under non-sink conditions.\textsuperscript{23,24,100} Sun et al. have introduced a dimensionless sink index (SI) to standardize the dissolution conditions:\textsuperscript{101}

\[
SI = \frac{c_{\text{cryst}} V}{\text{Dose}}
\]  

(1.4)

where \(c_{\text{cryst}}\) is the crystalline drug solubility, \(V\) is the volume of the dissolution media, and Dose is the total drug quantity. Most conventional USP protocols have SI > 3,\textsuperscript{100} while a perfect sink condition is achieved when the SI value > 10.\textsuperscript{101} On the other hand, it is considered as non-sink condition when SI < 0.1.\textsuperscript{100,101} For this dissertation, non-sink dissolution conditions are more relevant since all the ASD formulations are supposed to increase drug solubility at supersaturated concentrations, while drug permeability is not a big concern (i.e., BCS Class II compounds are used). Thus, all \textit{in vitro} dissolution tests performed in this dissertation are within non-sink conditions (e.g., SI = 0.09 for model drug phenytoin at 37 °C). Researchers have observed good \textit{in vitro} and \textit{in vivo} correlations using dissolution tests under non-sink conditions.\textsuperscript{100,102} For example, Ting et al. found that poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (poly(NIPAm-co-DMA)) with 70 mol % NIPAm composition was a top performing polymer to solubilize hydrophobic drugs, phenytoin and nilutamide, during an \textit{in vitro} dissolution
The results were consistent with an *in vivo* rat study, where phenytoin plasma concentration was maintained the highest over 6 h for poly(NIPAm-co-DMA) with 70 mol % NIPAm compared to copolymers with other NIPAm compositions.

**Figure 1.3.** In vitro dissolution tests of representative solid dispersions. Dissolution profiles of phenytoin and nilutamide show supersaturated drug concentration over time for drug only (×, dashed black) and formulations spray dried at 10 wt % (◇, solid dark orange) and 25 wt % (▽, solid dark green). Experiments were prepared with a total drug concentration of 1000 μg/mL. Error bars represent the range of collected data for N = 2. Figure adapted from Ting, J. M.; Tale, S.; Purchel, A. A.; Jones, S. D.; Widanapathirana, L.; Tolstyka, Z. P.; Guo, L.; Guillaudeu, S. J.; Bates, F. S.; Reineke, T. M. *ACS Cent. Sci.* 2016, 2, 748–755 (This is an open access article published under an ACS AuthorChoice License, https://pubs.acs.org/doi/abs/10.1021/acscentsci.6b00268). Copyright 2016 American Chemical Society, further permissions related to the material excerpted should be directed to the ACS.

Upon dissolution in aqueous solution, ASDs can rapidly disperse to form different species, which will affect the drug dissolution profiles (Figure 1.1). Molecularly dissolved “free” drugs can potentially undergo a fast exchange with drugs in the amorphous drug/polymer nanostructures (i.e., ~20-300 nm in size), resulting in an apparent solubility that is higher than the crystalline drug solubility. In contrast, large
amorphous particles (i.e., > 500 nm in size) and drug crystals will be separated from the supernatant by centrifugation during the \textit{in vitro} micro-centrifuge dissolution test.\textsuperscript{24} Therefore, it is important to characterize the colloidal particles existing in the dissolution media to better understand which species (i.e., molecularly dissolved drugs or drugs in form of the drug/polymer nanostructures) contribute to the apparent solubility in a typical \textit{in vitro} dissolution test.

Polarized light microscopy (PLM) and dynamic light scattering (DLS) are often used as complements to the dissolution test. PLM has been mainly used to directly detect drug crystals in solution,\textsuperscript{103,104} which will appear under polarized light due to their birefringent nature. The spatial resolution of PLM is approximately 200 nm, limited by half the wavelength of visible light.\textsuperscript{105} It is a straightforward method to visualize crystal growth, and to quantify crystal sizes in solution over time.\textsuperscript{106,107} For example, Alonzo and coworkers have observed the formation of dendritic needle-like indomethacin crystals directly from the solution phase in the absence of any polymers by PLM.\textsuperscript{103}

Dynamic light scattering (DLS) is a widely used experiment to quantify size distribution of particles in solution. Dalsin et al. have used DLS to investigate the size of the drug-polymer structures that led to enhanced ASD performance.\textsuperscript{108} It is a straightforward and powerful technique to characterize the different species during dissolution. However, one precaution of using DLS is that the particles to be analyzed must be stable over a period of time since the acquisition time for a reliable DLS measurement is at least a minute per angle. Once the ASDs are dissolved in aqueous solution, the drug-polymer structures are typically in a meta stable state and will most likely change over time. Therefore, the size quantification by DLS is not feasible if the size of these structures changes very rapidly during the acquisition time of the DLS measurement.
One way to resolve this limitation is to “freeze” the sample at different time points during the dissolution test. Recently, cryogenic transmission electron microscopy (cryo-TEM) has been used to enable a direct visualization of the drug-polymer nanoparticles with nanoscale spatial resolution, by vitrifying the dissolution media at different time points.\textsuperscript{11,54} Good correlation between a higher drug supersaturation concentration during dissolution and the existence of drug-polymer nanoparticles has been established in recent studies.\textsuperscript{11,54} In addition, by coupling electron diffraction with low-dose cryo-TEM, Ricarte et al. have shown that the crystalline and/or amorphous nature of the nanoparticles can be identified, as shown in Figure 1.\textsuperscript{4,54} Compared to DLS, direct visualization also allows the investigation of particle structure and morphology during dissolution, in addition to the particle size quantification. However, it is very time consuming to obtain cryo-TEM images (e.g., vitrification and imaging of one ASD sample at a specific time point during dissolution can easily take a few hours). It will be demonstrated in Chapter 5 that the drug dissolution profile is directly correlated with the nanoparticle structure and size evolution, and therefore the \textit{in situ} nanoparticle characterization enabled by cryo-TEM is important.
Figure 1.4. 25 wt % probucol SD dissolution media at (A) 4 min and (B) 360 min. (C) Low-dose cryo-TEM image and (D) electron diffraction pattern at 360 min. Electron diffraction pattern suggested nanoparticles are amorphous. Figure adapted from Ricarte, R. G.; Li, Z.; Johnson, L. M.; Ting, J. M.; Reineke, T. M.; Bates, F. S.; Hillmyer, M. A.; Lodge, T. P. *Macromolecules* **2017**, *50*, 3143–3152. Copyright 2017 American Chemical Society.

In parallel with the direct visualization from cryo-TEM in real space, small-angle X-ray scattering (SAXS) with a synchrotron source can also be used to quantify the sizes of the drug-polymer nanoparticles *in situ* using reciprocal space information. Despite the low electron density of organic compounds, the acquisition time for a reliable synchrotron SAXS experiment is a few seconds. Thus, real-time changes in the species existing in aqueous solution during the dissolution tests can be captured. The short acquisition time will enable early time-point investigation of the structures during the dissolution test. Moreover, the SAXS patterns can also give information about nanoparticle morphology. For example, Ricarte el al. found that some of their SAXS patterns fit better to a triaxial ellipsoid form factor than a spherical form factor, which is
consistent with the irregular shapes observed from cryo-TEM. One limitation of the SAXS experiment is that it may not capture the entire form factor for nanoparticles with a size $\gtrsim 100$ nm to obtain a reliable size distribution, due to the scattering vector ($q$) range constraint of the instrument. SAXS and cryo-TEM can be used in combination as complements to DLS and PLM, to fully understand the solution-state properties (e.g., drug crystallization and evolution of drug-polymer nanoparticles) during the in vitro dissolution test.

Drug-polymer interactions in solution can often dictate the existence of drug-polymer nanoparticles. Furthermore, the strength of any drug-polymer partitioning will significantly affect the inhibition of drug nucleation and crystal growth. Therefore, it is important to investigate and ultimately quantify the partitioning of drug molecules to polymers in solution. Nuclear magnetic resonance (NMR) spectroscopy has been used to probe the intermolecular interactions between drug and polymer in a supersaturated solution. Chen and coworkers have used solution-state $^{13}$C NMR spectroscopy to determine drug-polymer interactions, where the hydrogen bonding between the NH group of felodipine and the C=O group of PVP is clearly illustrated by related carbon peak shifts in the spectra. Ueda and coworkers have used 2D $^1$H nuclear Overhauser effect spectroscopy (NOESY) to probe the intermolecular interactions between carbamazepine (CBZ) and HPMCAS in solution, as shown in Figure 1.5. The cross peaks between the peaks of carbamazepine around 7.0–8.0 ppm and those of HPMCAS peaks at 1.0–4.0 ppm clearly indicate the close proximity of drugs and polymers in solution. In parallel, Raman spectroscopy can also be used in solution to detect specific intermolecular interactions, as a complement to NMR spectroscopy. For example, Raina et al. collected the Raman spectra of amorphous nifedipine in PBS solution by using a fiber optically coupled noncontact probe, successfully identifying changes of the drug polymorph over time. Although integration of the drug-polymer cross peaks with respect to other cross
peaks (e.g., drug-drug peaks) can provide some insights to the strength of the drug-polymer interactions, NOESY and Ramen are more commonly used for a qualitative detection of the specific interactions.

![Figure 1.5. NOESY spectrum of carbamazepine (CBZ, 800 μg/mL) and HPMCAS (HF, 3200 μg/mL) solution at a mixing time of 1.5 s. Figure adapted from Ueda, K.; Higashi, K.; Yamamoto, K.; Moribe, K. Mol. Pharm. 2013, 10, 3801–3811. Copyright 2013 American Chemical Society.](image)

Pulsed-field gradient (PFG) NMR, enables a direct quantification of the drug-polymer “binding” constant by measuring the small molecule diffusivity in the presence and absence of polymers.\(^\text{11,55}\) So et al. previously used PFG-NMR to probe small molecule exchange through macromolecular structures such as the bilayer membranes of nanoemulsion-like polymersomes, as illustrated in Figure 1.6.\(^\text{112}\) When the exchange rate
is slow relative to the diffusion time of the PFG-NMR experiment (i.e., on the order of 100 ms), the echo decay curve can be fit to a biexponential function, from which the diffusion coefficients and mole fractions of the two species (i.e., “free” small molecules and small molecules associated with the macromolecular structures) can be obtained.\textsuperscript{112} In contrast, when the exchange time between the free and “bound” small molecules is much shorter than the diffusion time of the PFG-NMR experiment (i.e., fast exchange), the decay curve can be described by a single exponential function, resulting in a time-weighted diffusion coefficient that represents the number-average diffusivity of the two species.\textsuperscript{11,55} It is worth noting that a different small molecule tracer (e.g., 3-(trimethylsilyl)propionic-2,2,3,3-d\textsubscript{4} acid sodium salt, TSP), in addition to the model drug, is often added as a control in these experiments,\textsuperscript{11,55} to ensure that any measured changes in diffusion of the small molecules were not caused by changes in the macroscopic solution viscosity with added polymer.\textsuperscript{113} The combination of NOESY and PFG-NMR experiments provides molecular-level understanding of a drug-polymer interactions, and therefore can potentially enable a rational design of the polymer structures for different model drugs in the future. It will be demonstrated in Chapters 3 and 4 that these two 2D NMR experiments are simple yet powerful tools to investigate drug partitioning to various polymer structures (e.g., free chains vs. micelles).
Figure 1.6. (a) Schematic of molecular diffusion in each phase and transportation across the membrane of polymersome with ionic liquid interior dispersed in water. The tracer molecules move with diffusion coefficient $D_i$, inside and $D_e$ outside, of the polymersomes. Also, the molecules are exchangeable across the membranes through paths 1 and 2. (b) Experimental data and fitted echo curve of the proton from 1-ethyl-3-methylimidazolium ([EMIM]) in the polymersome solution with various diffusion times. Figure adapted from So, S.; Lodge, T. P. J. Phys. Chem. C 2014, 118, 21140–21147. Copyright 2014 American Chemical Society.

1.5 Dissertation Outline

The goals of this project are to design well-defined polymers as model systems to solubilize hydrophobic drug molecules, and more importantly, to elucidate the drug solubility and dissolution enhancement mechanism. Altogether, three different strategies have been used to solubilize hydrophobic drugs for oral delivery: polymer blends, micelle-forming diblock polymers, and chemically crosslinked polymer nanogels. Almost all the polymers in this project were synthesized in our lab so that their physical and chemical properties (i.e., monomer structure/composition, end group, molecular weight, morphology) can be precisely tuned. We aim to formulate the specialized polymers together with a variety of hydrophobic model drugs, in which the control over nano- and micro-structure can result in good understanding of the drug dissolution performance. The correlation between the macromolecular design and the performance of the drug-
polymer formulation can be discovered and translated to rational design of effective polymeric excipients for oral drug delivery. The above-mentioned characterization techniques in both solid and solution states were used together to better understand the structure-property relationships and elucidate the underlying drug solubility enhancement mechanism.

Chapter 2 describes the details of the experimental techniques used throughout this dissertation, including polymer synthesis and characterization, drug-polymer interaction characterization, and ASD characterization in both solid and solution states.

In Chapter 3, we discover that a strategy of using polymer blends can achieve better performance (i.e., in terms of prolonged maintenance of high super-saturation of drug) than either component alone. This is due to the separate demands of rapid dissolution (the so-called “spring”) and inhibition of drug crystallization (the “parachute”). Second, and most important, we show that inhibition of drug crystallization is favored by forming micelles in which the drug is sequestered in the slightly hydrophobic corona region. We propose that a locally concentrated polymer environment (i.e., micelle corona) favors hydrophobic drug partitioning to the polymer, and therefore results in enhanced drug dissolution performance. This mechanism is entirely distinct from the standard approach of using micelles for drug delivery, in which the drug is sequestered in the core.

Chapter 4 is an extension of Chapter 3, in which we design diblock polymers that form micelles with crowded corona regions. The corona density of the polymer micelle was tuned by varying the corona block length while keeping the core block length constant. In this work, the drug sequestration strategy discovered in Chapter 3 was applied to two different hydrophobic model drugs, phenytoin and nilutamide, and such a strategy was shown to be effective. Furthermore, as many drug-polymer formulations result in metastable states, we showed that the drug loading method (i.e., forming
micelles before or after the spray drying process) also plays a critical role to dictate the drug dissolution performance.

In Chapter 5, instead of using a physical way to produce polymer micelles as in Chapters 3 and 4, we took a different approach and chemically crosslinked polymers to form nanogels with a locally concentrated environment for hydrophobic drug solubility enhancement. These chemically crosslinked nanogels served as reservoirs to solubilize the drug by inhibiting drug nucleation and crystal growth. Nanoparticles, presumably nanogels with encapsulated drug molecules, were directly visualized and quantified during the dissolution process of the ASDs by a combination of cryo-TEM and SAXS. The drug dissolution performance was highly correlated with the evolution of these drug-nanogel particles.

Chapter 6 presents a summary of the key findings of the previous chapters. In addition, based our current understanding of the underlining mechanism discovered in this dissertation, we propose a few directions to move forward in this project and to inspire future innovations.
2. Experimental Techniques

This chapter describes the experimental techniques used in Chapters 3-5. Two polymerization techniques, reversible addition-fragmentation chain transfer (RAFT) and emulsion polymerizations, will be introduced briefly and the experimental details will be provided. A variety of polymer characterization techniques will be discussed including size-exclusion chromatography (SEC), nuclear magnetic resonance spectroscopy (NMR), light scattering, optical transmittance, and viscometry. Two 2D $^1$H NMR techniques, nuclear Overhauser effect spectroscopy (NOESY) and diffusion-ordered spectroscopy (DOSY, also known as pulsed field gradient NMR), are used in this thesis to investigate drug-polymer interactions. The polymers and model drugs are spray dried into solid dispersions, which are characterized by a set of techniques in both solid and solution states, including scanning electron microscopy (SEM), wide-angle X-ray scattering (WAXS), differential scanning calorimetry (DSC), *in vitro* dissolution assay, polarized light microscopy (PLM), cryogenic transmission electron microscopy (cryo-TEM), and small-angle X-ray scattering (SAXS).

2.1 Polymer Synthesis

Reversible Addition-Fragmentation Chain Transfer Polymerization

Controlled radical polymerization ranked first in the “Top Ten Achievements” of the polymer field over the past five decades.$^{114}$ It has the advantages of both conventional radical polymerization and “living” polymerization; the former only requires relatively relaxed polymerization conditions and works with many monomers, while the latter provides narrow molecular weight distributions and defined end group functionality.$^{115}$ As one of the most commonly used controlled radical polymerization techniques, reversible addition-fragmentation chain transfer (RAFT) polymerization, has now been
adopted by a broad community to produce their own polymeric materials for both fundamental research in academia and specialized applications in industry, since its first report in 1998.\textsuperscript{116,117}

As its name implies, the most important component of RAFT polymerization is the chain transfer agent (CTA). The CTA, typically a dithioester or trithiocarbonate, allows the radical species to equilibrate between active and dormant species. Since the propagation rate is often much slower than the rate of addition/fragmentation equilibrium, polymer chains grow at approximately the same rate, resulting in a similar degree of polymerization (DP) at a given time, and therefore, narrow molecular weight distribution.\textsuperscript{117,118} The polymer end-group functionality is dependent on the type of CTA used, which can either be cleaved or remain to obtain the desired structures and morphologies.\textsuperscript{13,119,120} For materials described in Chapters 3 and 4, two types of CTA with different length of hydrocarbons were used: S-1-dodecyl-S-(\(\alpha,\alpha'\)-dimethyl-\(\alpha''\)-acetic acid) trithiocarbonate (C\textsubscript{12}) and 4-cyano-4-[(ethylsulfanylthiocarbonyl) sulfanyl] pentanoic acid (C\textsubscript{2}).

Using Chapter 4 as an example, a series of three poly(N-isopropylacrylamide-\textit{co}-N,N-dimethylacrylamide)-\textit{b}-polystyrene (PND-\textit{b}-PS-C\textsubscript{12}) diblock polymers were synthesized by a two-step RAFT polymerization using the same macro-CTA, poly(N-isopropylacrylamide-\textit{co}-N,N-dimethylacrylamide) (PND-C\textsubscript{12}), according to procedures adapted from the literature,\textsuperscript{116,121,122} as shown in Figure 2.1. A second statistical copolymer, PND-C\textsubscript{2}, targeting the same molecular weight and NIPAm mole fraction of the PND-C\textsubscript{12}, was synthesized using a different CTA (C\textsubscript{2}) according to the protocol shown in Figure 2.2. These polymers are designated as PND\textsubscript{34}-C\textsubscript{2}, PND\textsubscript{34}-C\textsubscript{12}, PND\textsubscript{34}-\textit{b}-PS\textsubscript{2}-C\textsubscript{12}, PND\textsubscript{34}-\textit{b}-PS\textsubscript{9}-C\textsubscript{12}, and PND\textsubscript{34}-\textit{b}-PS\textsubscript{14}-C\textsubscript{12}, with the subscripts denoting the \(M_n\) of the corresponding block in kg/mol and the suffix denoting the type of chain end based on the CTA used.
Figure 2.1. Synthesis of poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PND-C_{12}) and poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-polystyrene (PND-b-PS-C_{12}) via RAFT polymerization.

Figure 2.2. Scheme of the RAFT polymerization of poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PND-C_{2}).

PND_{34}-C_{12} macro-CTA, a statistical copolymer, was first synthesized via RAFT polymerization by using NIPAm and DMA as comonomers. NIPAm (20.0 g, 0.177 mol), DMA (7.50 g, 0.0757 mol), CTA S-1-dodecyl-S-(α,α’-dimethyl-α”-acetic acid) trithiocarbonate (C_{12}) (0.307 g, 0.842 mmol) and recrystallized AIBN (0.0138 g, 0.0840 mmol) were combined with 126 mL of 1,4-dioxane in a Schlenk flask. Three freeze-pump-thaw cycles were performed, after which the flask was filled with argon and removed from the vacuum line. The reactants were stirred at 72 °C for 4 h and then the reaction was quenched by placing the flask into an ice bath. The reaction product was
dissolved with extra THF and precipitated by dropwise addition to ice-cold hexanes, followed by filtration. The polymer was redissolved in THF and precipitated a second time into hexanes, filtered, and dried in a vacuum oven at approximately 50 °C for 24 h.

PND-b-PS-C_{12} diblock polymers were synthesized following a similar procedure, using the PND_{34}-C_{12} macro-CTA. PND_{34}-C_{12} (3.50 g, 0.104 mmol), styrene (2.69 g, 0.0259 mol) and recrystallized AIBN (1.7 mg, 0.010 mmol) were combined with 10 mL of THF in a Schlenk flask. After three freeze-pump-thaw cycles, the flask was filled with argon. The reaction was allowed to run at 80 °C for 18 h and then quenched by placing the flask in an ice bath. The polymer was dissolved in extra THF and precipitated dropwise in ice-cold hexanes, followed by filtration; this procedure was repeated. The final product was dried in a vacuum oven at approximately 50 °C for 24 h and stored in a desiccator under reduced pressure until use.

**Emulsion Polymerization**

Emulsion polymerization is one of the most commonly used techniques to prepare monodisperse aqueous latex dispersions, also known as microgels or nanogels.\textsuperscript{123,124} First synthesized by Staudinger and Husemann in 1935,\textsuperscript{125} microgels have been studied extensively and their preparation, properties, theory, and applications are discussed comprehensively in previous reviews.\textsuperscript{126-128} Microgels with narrow size distribution can often be produced with emulsion polymerization, and the particle size can be tuned by varying the amount of surfactant. All the nanogels in Chapter 5 were synthesized by emulsion polymerization and the experimental details are described below.

A series of three poly(\(N\)-isopropylacrylamide) (PNIPAm) nanogels with varying crosslink density (0.5, 2.5, and 5.0 mol\% of \(N,N'\)-methylenbis(acrylamide) (BIS)) was synthesized by emulsion polymerization according to procedures adapted from the
literature,\textsuperscript{129,130} as illustrated in Figure 2.3. In a typical emulsion polymerization targeting 0.5 mol% crosslink density, NIPAm (8 g, 0.0708 mol), BIS (0.0545 g, 0.35 mmol), and sodium dodecyl sulfate (SDS) (0.15 g, 0.52 mmol) were dissolved in 400 mL of Milli-Q water in a three-neck flask equipped with a reflux condenser, a gas inlet, and a magnetic stir bar. The aqueous solution was bubbled with nitrogen gas under constant stirring of 300 rpm at 72 °C for at least 1 h. The initiator, potassium persulfate (KPS) (0.3 g, 1.1 mmol), was dissolved in 40 mL of Milli-Q water and bubbled with nitrogen gas for 1 h. Then, the KPS solution was cannula-transferred to the three-neck flask to initiate the polymerization. The reactants were stirred at 300 rpm at 72 °C for 5 h, after which the dispersion was cooled with an ice bath and filtered through glass wool. The solution was purified by extensive dialysis against water for 14 d, lyophilized, and further dried in a vacuum oven at approximately 50 °C for at least 48 h.

![Reaction scheme for the emulsion polymerization of N-isopropylacrylamide (NIPAm) in aqueous solution.](image)

**Figure 2.3.** Reaction scheme for the emulsion polymerization of N-isopropylacrylamide (NIPAm) in aqueous solution.\textsuperscript{131}

Three poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PND) nanogels with a constant crosslink density (5.0 mol% BIS relative to total monomer) but varying N,N-dimethylacrylamide (DMA) compositions were synthesized following a similar procedure. In a typical emulsion polymerization of PND nanogel targeting 40 mol%
DMA, NIPAm (0.631 g, 5.6 mmol), DMA (0.369 g, 3.72 mmol), BIS (0.0716 g, 0.46 mmol), and SDS (0.0188 g, 0.065 mmol) were dissolved in 50 mL of Milli-Q water, and stirred at 300 rpm at 72 °C. KPS (0.004 g, 0.015 mmol) was dissolved in 5 of Milli-Q water. Both solutions were bubbled with nitrogen gas for 1 h. The KPS solution was then cannula-transferred to the reactant solution and the reaction was allowed to run at 72 °C while stirring for 5 h. The final dispersion was purified and dried as described above.

2.2 Polymer Characterization

Size-Exclusion Chromatography

The molecular weight distributions of the polymers were determined by size-exclusion chromatography (SEC), on an Agilent 1260 Infinity series system with a Wyatt Dawn Heleos II multiangle laser light scattering (MALS) detector and a Wyatt Optilab T-rEX refractive index detector with THF as the eluent at 25 °C. Using Chapter 4 as an example, a weight-averaged refractive index increment (dn/dc) of 0.0953 mL/g was used for PND statistical copolymers in THF, based on the composition obtained from the 1H NMR spectra and literature dn/dc values of PNIPAm (0.107 mL/g) and PDMA (0.068 mL/g) in THF.132,133 SEC traces of the PND and PND-b-PS polymers are shown in Figure 2.4. The number-average molecular weight ($M_n$) and dispersity ($D$) of the synthesized polymers are summarized in Table 2.1.
Figure 2.4. SEC curves of the polymers (in THF) synthesized by RAFT polymerization. Solid lanes and dot lanes represent normalized intensities from the refractive index (RI) and light scattering (LS) detectors, respectively.

Table 2.1. Polymer characteristics

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_n$ of PND$^a$ (kg/mol)</th>
<th>$M_n$ of PS$^b$ (kg/mol)</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND$_{34}$-C$_2$</td>
<td>34</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>PND$<em>{34}$-C$</em>{12}$</td>
<td>34</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>2$-C$</em>{12}$</td>
<td>34</td>
<td>2.0</td>
<td>1.09</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>9$-C$</em>{12}$</td>
<td>34</td>
<td>8.8</td>
<td>1.16</td>
</tr>
<tr>
<td>PND$<em>{34}$-b-PS$</em>{14}$-C$_{12}$</td>
<td>34</td>
<td>14</td>
<td>1.21</td>
</tr>
</tbody>
</table>

a. based on SEC-MALS data (using weight-averaged $dn/dc = 0.0953$ mL/g)  
b. based on $^1$H NMR spectroscopy  
All PNDs have 67 mol% of NIPAm (based on $^1$H NMR spectroscopy).

Nuclear Magnetic Resonance Spectroscopy

Polymer compositions were assessed by $^1$H nuclear magnetic resonance (NMR) spectroscopy, on a Bruker Avance III HD (500 MHz) spectrometer (equipped with a 5 mm Prodigy TCI cryoprobe) in deuterated solvent at 25 °C. Using Chapter 4 as an example, the compositions of NIPAm for PND$_{34}$-C$_2$ and PND$_{34}$-C$_{12}$ were both 67 mol%,
based on $^1$H NMR analysis shown in Figure 2.5 (i.e., molar ratio of protons e to proton d was 2.985 by integrating the NMR spectrum). The polystyrene compositions for PND-$b$-PS-C$_{12}$ diblock polymers were also calculated from $^1$H NMR spectra (Figure 2.5). Polystyrene molecular weight for the PND-$b$-PS-C$_{12}$ diblock polymers was determined based on the PND molecular weight from SEC and the polystyrene composition from $^1$H NMR. Using PND$_{34}$-$b$-PS$_9$-C$_{12}$ as an example, molar ratio of protons a to proton d was 1.934 by integrating the NMR spectrum, and therefore the polystyrene molecular weight was calculated to be 8.8 kg/mol, as shown by sample calculation in Equation 2.1.

$$M_{n,PS} = 34 \text{ kg/mol} \cdot \frac{1.934}{5} \cdot 0.67 = 8.8 \text{ kg/mol}$$  \hspace{1cm} (2.1)

Figure 2.5. $^1$H NMR spectra of the polymers in deuterated chloroform (CDCl$_3$).
Light scattering

Both dynamic (DLS) and static light scattering (SLS) were performed in Chapters 3-5 using a Brookhaven BI-200SM system with a 637 nm laser at 22 °C. Polymers were dissolved directly in a PBS (pH=6.5) solution and passed through a filter to remove dust before transferring into a glass tube or a scintillation vial for DLS and SLS measurements, respectively. For DLS, the autocorrelation function of the scattered light intensity was obtained at scattering angles from 60° to 120° in 15° increments, with a typical count rate of 200 kilocounts/s and acquisition time of 10 min per angle. Data were first analyzed using the Laplace inversion routine REPES\textsuperscript{134} to obtain the hydrodynamic radius ($R_h$) distribution. The same autocorrelation functions were then fit with either a second-order cumulant model to obtain the mean hydrodynamic radius $R_h$ and dispersity ($\mu_2/\Gamma^2$), or a double exponential decay model yielding two distinct $R_h$ values. Using Chapter 4 as an example, the normalized autocorrelation functions ($g^{(2)}$) of the scattered light intensity at scattering angles from 60° to 120° in 15° increments were fitted to a second-order cumulant model for PND$_{34}$-b-PS$_{14}$-C$_{12}$, defined as:

$$g^{(2)}(\tau) = B + \beta e^{-2\Gamma \tau} (1 + \frac{\mu_2 \Gamma^2}{2})^2$$

where $\Gamma$ is the mean decay rate, $\tau$ is the delay time, $B$ is the baseline (long-time value of $g^{(2)}$), $\beta$ is amplitude of the decay, and $\mu_2$ is the second moment of the decay rate distribution. Representative autocorrelation functions along with the best fits to Equation 2.2 and the corresponding decay rate $\Gamma$ versus $q^2$ plot are shown in Figure 2.6. The linear dependence of the decay rates on $q^2$ and the low value of the intercept both confirm that the relaxation is diffusive.
Figure 2.6. Examples of (a) autocorrelation functions and (b) decay rate $\Gamma$ versus $q^2$ for 10 mg/mL PND$_{34}$-b-PS$_{14}$-C$_{12}$ in PBS solution at 37 °C, using a second-order cumulant fit.

For SLS, the molecular weight ($M_w$) of the polymer (as free chains or as micelles) and the second virial coefficient ($A_2$) were calculated from the Zimm equation:

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} \left(1 + \frac{1}{3} q^2 R_g^2\right) + 2A_2c + \cdots$$  \hspace{1cm} (2.3)

where $K$ is the optical factor, $R_\theta$ is the Rayleigh ratio, $R_g$ is the radius of gyration, $q$ is the scattering vector, and $c$ is the polymer concentration. Using Chapter 4 as an example, $dn/dc$ values of the samples were measured on a SEC instrument (Agilent, Santa Clara CA) with an Optilab rEX refractometer ($\lambda = 658$ nm, Wyatt Technologies, Santa Barbara, CA) using PBS solution (pH = 6.5) as the eluent, as shown in Figure 2.7 and Table 2.2.
Figure 2.7. dn/dc measurements of PND and PND-b-PS in PBS buffer (pH = 6.5). The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>dn/dc (mL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND$_{34}$-C$_2$</td>
<td>0.164</td>
</tr>
<tr>
<td>PND$<em>{34}$-C$</em>{12}$</td>
<td>0.165</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>2$-C$</em>{12}$</td>
<td>0.172</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>9$-C$</em>{12}$</td>
<td>0.176</td>
</tr>
<tr>
<td>PND$<em>{34}$-b-PS$</em>{14}$-C$_{12}$</td>
<td>0.187</td>
</tr>
</tbody>
</table>

Table 2.2. Summary of the measured dn/dc

PND copolymers and lyophilized PND-b-PS-C$_{12}$ micelles were dissolved in PBS solution (pH = 6.5) and passed through 0.2 μm filters into clean glass tubes for measurements. Scattering intensity was monitored between angles from 60 to 120°. The Rayleigh ratio of the samples was calibrated using a filtered ultrapure (≥ 99.9%) toluene standard ($R_0 = 1.363 \times 10^{-5}$ cm$^{-1}$). Figure 2.8 is a representative Zimm plot of the PND$_{34}$-C$_{12}$ in PBS buffer (pH = 6.5) at 27 °C. The $M_w$, $R_g$, and $A_2$ were determined to be $3.1 \times 10^5$ g/mol, $22 \pm 5$ nm, and $7.1 \times 10^{-5}$ mol cm$^3$/g$^2$, respectively.
In Chapter 3, assuming scattering in the Rayleigh regime ($qR_g << 1$), the weight average molecular weight ($M_w$) of the polymer (as unimers or micelles) and the second virial coefficient ($A_2$) were calculated from the simplified Zimm equation (for structures with relatively small $R_0$ values such as free chains and self-assembled micelles):

$$\frac{Kc}{R_0} = \frac{1}{M_w} + 2A_2c + \cdots$$ (2.4)

where $K$ is the optical factor, $R_0$ is the Rayleigh ratio, and $c$ is the polymer concentration. The literature refractive index increment (dn/dc) of 0.167 mL/g was used for PNIPAm in aqueous solution. For both DLS and SLS, the measured PNIPAm concentrations (1–9 mg/mL) were equivalent to the ones used in the in vitro dissolution study and lower than the chain overlap concentration ($c^*$) calculated based on previous reports.
Optical Transmittance

Optical transmittance is used to determine the cloud point of a polymer in aqueous solution. Polymers were directly dissolved in PBS solution (pH=6.5) at desired concentrations and transferred to a glass ampule, which was then incubated in a heating stage with temperature control (± 1 °C). A 10 mW helium-neon laser (λ = 633 nm) was aligned to pass through a neutral density filter and then the glass ampule. The transmitted light was focused by a lens onto a photodiode detector for data collection. All measurements were performed with a heating rate of 0.25 °C/min.

Viscometry

Viscosities of the microgel and pure PBS solutions were measured in duplicate with a Cannon-Fenske viscometer tube (size 25, Sigma Aldrich) in a temperature-controlled (± 0.1 °C) water bath at 22 and 37°C. Figure 2.9 is an image taken during the viscosity measurement to demonstrate the instrument setup.

Figure 2.9. Viscometry instrument setup.
2.3 Characterization of Drug-Polymer Interactions in Aqueous Solution

Nuclear Overhauser Effect Spectroscopy

2D $^1$H NOESY experiments were performed on a Bruker Avance III HD (500 MHz) instrument with a 5 mm Prodigy TCI cryoprobe at 25 °C. The pulse sequences “noesygpphpp” (NOESY with gradient pulses during mixing time, phase sensitive, and purge pulses) or “noesyesgpph” that employed excitation sculpting with gradients for water suppression, were employed with a relaxation delay of 3 s, a mixing time of 1.5 s (unless otherwise noted), and 2048 data points × 256 increments × 8 scans. All spectra were aligned with the TSP protons (0 ppm). Data were analyzed with the Topspin software package.

Diffusion-Ordered Spectroscopy

Diffusion-Ordered Spectroscopy (DOSY), also known as pulse field gradient (PFG) NMR, is a technique used to measure the diffusion coefficients of small molecules (e.g., model drugs) and polymers in aqueous solution. 2D $^1$H DOSY experiments were performed on a Bruker Avance III (500 MHz) instrument with a 5 mm Broadband Fluorine Observe (BBFO) probe at 27 °C to determine the diffusion coefficients of phenytoin, TSP, and HPMCAS or PNIPAm at various polymer concentrations. The pulse sequence “ledbpgp2s” (longitudinal eddy current delay experiment using bipolar gradients acquired in 2D) was employed with a relaxation delay of 10 s and a total of 32 scans. Data were analyzed with the Topspin software package and the diffusion coefficient ($D$) of each species was extracted from fits to Equation 2.5:

$$\ln \left( \frac{I(g)}{I(0)} \right) = -\gamma g \delta^2 D(\Delta - \frac{\delta}{3})$$  \hspace{1cm} (2.5)
where \( I(g) \) is the recorded echo intensity at various gradient strengths (\( g \), from 2 to 95% of the maximum strength), \( \gamma \) is the gyromagnetic ratio of \(^1H\), \( \delta \) is the length of the gradient pulse (2 ms), and \( \Delta \) is the diffusion time (100 ms).

### 2.4 Solid Dispersions Preparation and Solid-State Characterization

#### Spray Drying

Spray-dried dispersions (SDDs) were prepared at 10 or 25 wt% drug loading. Polymers and model drugs were both dissolved in methanol or THF with a total solids concentration of 2 wt%. This solution was transferred into a 20 mL syringe and injected into a mini spray dryer (Bend Research) with a flow rate of 0.65 mL/min. The nitrogen gas flow rate and inlet temperature were set at 12.8 standard L min\(^{-1}\) and 72 °C, respectively. SDDs were collected from the filter paper, transferred into a scintillation vial, and dried under reduced pressure at ambient temperature for at least 24 h. The prepared samples were stored in a desiccator under reduced pressure until use.

#### Scanning Electron Microscopy

In Chapter 3, SDDs were deposited on carbon tape and sputter-coated with \( \sim 10 \) nm of conductive gold/palladium (60/40 w/w) coating by a Denton DV-502A High Vacuum Deposition System. In Chapter 5, SDDs were deposited on carbon tape and coated with \( \sim 10 \) nm of iridium coating by a Leica EM ACE600 sputter coater. In both cases, the samples were imaged using a Hitachi S-4700 high-resolution field-emission-gun scanning electron microscope at an accelerating voltage of 1.5 or 3.0 kV. Images were taken at a long working distance of \( \sim 18 \) mm, a tilt angle of 45° towards the lower secondary electron (SE) detector, and a magnification from 5000× to 10000×.
**Wide-Angle X-ray Scattering**

WAXS data were collected using either a lab-scale source or a synchrotron source at the Argonne National Laboratory. Lab-scaled WAXS experiments were performed on a Bruker-AXS (Siemens) D5005 Diffractometer with a 2.2 kW sealed Cu source (λ = 1.54 Å). Approximately 50 mg of the SDDs were loaded on a standard glass holder and inserted into the sample chamber. All experiments were taken at a voltage of 45 kV and current of 40 mA with a 20° angle range from 5° to 40° (0.02° increments). Synchrotron WAXS experiments were performed on the DND-CAT 5-ID-D beamline at the Advanced Photon Source, Argonne National Laboratory. SDDs were loaded into nylon sleeve washers and sealed with Kapton tape. The washers were placed in a holder at room temperature (approximately 25 °C). 2D scattering patterns were collected on a Rayonix CCD area detector with an exposure time of 1 s, and azimuthally integrated into 1D data of intensity (arbitrary units) vs scattering vector q (Å⁻¹). Background was removed by subtracting the empty washer sealed with Kapton tape reference from the 1D data of the SDD samples.

**Differential Scanning Calorimetry**

DSC experiments were collected using a Discovery DSC instrument (TA Instruments). SDDs were weighted and transferred into aluminum pans with standard lids. For all the SDDs, the first heating curve results are reported to analyze the material as prepared by spray drying, and to avoid any potential phase separation or crystallization induced by heating. The samples were heated from 0 °C to 160 °C with the following parameters: heating rate of 1 °C/min, modulated temperature amplitude of 1 °C, and modulated temperature period of 40 s.
2.5 Solution-state Characterization of Solid Dispersions

*In Vitro Dissolution Assay*

The *in vitro* dissolution test was performed in duplicate at 22 °C or 37 °C using the microcentrifuge method under non-sink conditions.23,24 SDDs were weighed into 2.0-mL conical microcentrifuge tubes. PBS solution (pH = 6.5) with 0.5 wt% of FaSSIF was added to the SDDs to reach a desired drug concentration of 1000 μg/mL (i.e., either 7.2 mg of SDD with 25 wt% drug loading or 18.0 mg of SDD with 10 wt% drug loading were dissolved in 1.8 mL of PBS/FaSSIF solution). The microcentrifuge tubes were vortexed for 1 min and placed in a VWR Analog dry heating block at 22 °C or 37 °C. At 4, 10, 20, 40, 90, 180, and 360 min, the dissolution medium was centrifuged for 1 min at 13000 rpm, and then a 50 μL aliquot of the supernatant was collected in a HPLC vial and diluted with 250 μL of MeOH. The microcentrifuge tubes were then vortexed for an additional 30 s, returned to the heating block and held at 37 °C until the next time point. The samples were outside the heating block during the aliquot collection process for approximately 2 min.

Solubilized drug concentration (i.e., phenytoin or nilutamide) in the supernatant was analyzed by reverse-phase high-performance liquid chromatography (HPLC) using a 1260 Infinity Quaternary Liquid Chromatograph System with a reversed-phase EC-C18 column (Poroshell 120, 4.6 × 50 mm, 2.7 μm, Agilent) and an Infinity Multiple Wavelength UV-Vis Detector. The mobile phase was 60:40 and 50:50 (v/v) of water/acetonitrile solution at a flow rate of 1 mL/min for SDDs containing phenytoin or nilutamide, respectively. For both drugs, calibration curves were developed at a wavelength of 240 nm to determine the sample concentration. Equilibrium solubility of the drug was determined by dissolving an excess amount of crystalline phenytoin or nilutamide in the PBS/FaSSIF solution, and equilibrating at 22 °C or 37 °C for 48 h. The
sample was centrifuged for 1 min at 13000 rpm to remove undissolved drug and the supernatant concentration was analyzed by HPLC using the same conditions as described above.

**Polarized Light Microscopy**

The dissolution medium of the SDDs was examined by polarized light microscopy (PLM) to directly visualize drug nucleation and crystal growth in a PBS/FaSSIF solution during dissolution at 37 °C. At each time point during the *in vitro* dissolution test described above, the solution was further vortexed for 30 s and a 20 μL aliquot was pipetted onto a pre-cleaned glass microscope slide (3” × 1”, Gold Seal) and the solution droplet was immediately covered with a glass microscope coverslip (24 × 40 mm, Fisher). Images were taken in triplicate using a Nikon Optiphot polarized light microscope at 10× magnification and recorded with a Canon SL1 digital camera. Observation of the phenytoin crystals under polarized light was facilitated by their birefringence. Particle size analysis was performed on each image after background subtraction and threshold adjustment using ImageJ software (National Institutes of Health, MD, USA) to obtain the number and radius of the observed crystals (*i.e.*, estimated as spheres for size quantification).

**Cryogenic Transmission Electron Microscopy**

The morphology and size of the nanostructures for polymers or the SDDs in the dissolution media were imaged by cryo-TEM in PBS solution during dissolution at 22 °C or 37 °C. Following the procedure of the *in vitro* dissolution study, the solution was further vortexed for 30 s at each time point before vitrification. For each specimen, a 2 μL solution was extracted from the dissolution medium and pipetted onto a 300-mesh carbon-coated lacey Formvar TEM grid (Ted Pella) inside a FEI Vitrobot Mark III vitrification system held at 26 °C and 100% relative humidity. The specimen was blotted
for 5-7 seconds with an offset of –2 mm, allowed to relax for 3 s, and plunged into a reservoir of liquid ethane (approximately 10-20 s). The vitrified grid was then stored in liquid nitrogen and transferred into a Gatan 626 cryo sample holder and imaged by an FEI Tecnai G2 Spirit BioTWIN with an accelerating voltage of 120 kV at approximately –177 °C. The images were recorded by an Eagle 2k (4 megapixel) CCD camera at a magnification range from 20000× to 40000× with an underfocus of 5–15 μm.

**Small-angle X-ray scattering**

SAXS experiments were performed on the DND-CAT 5-ID-D beamline at the Advanced Photon Source, Argonne National Laboratory. SDDs were loaded into 3.7 mL glass vials and PBS solutions (pH=6.5) were added to achieve the desired phenytoin concentration (t = 0 min). These vials were vortexed for 1 min and incubated in an aluminum vial holder at 22 °C or 37 °C. At various time points (t = 10, 20, 40, 90, 180, and 360 min), an approximately 0.1 mL aliquot of dissolution media was collected after 30 s of vortexing and transferred into 1.5 mm quartz capillary tubes. The vials were returned to the aluminum holder and incubated at 22 °C or 37 °C until the next time point. Polymer samples were prepared and transferred directly into the capillary tubes. The capillary tubes were placed in a multi-capillary holder at the desired temperature. Isotropic 2D scattering patterns were collected immediately after the closure of the hutch door on a Rayonix CCD area detector with an exposure time of 1-3 s, and azimuthally integrated into 1D data of intensity (arbitrary units) vs scattering vector q (Å⁻¹). Background was removed by subtracting the PBS solvent reference from the 1D data of the polymer microgel and dissolution media solution. The resulting intensity data were analyzed using the SASView software.
3. Synergistic Action of Polymer Blends


3.1 Introduction

The use of a solid dispersion, a mixture of drug dispersed in a solid polymer excipient matrix, can be an effective way to enhance the bioavailability of poorly water-soluble drugs. Kinetically stabilizing the drug in an amorphous state increases solubility, often by orders of magnitude. In this regard, hydroxypropyl methylcellulose acetate succinate (HPMCAS) has been shown to be a promising excipient for a variety of drugs. However, after an initial release, HPMCAS fails to maintain drug supersaturation for many hydrophobic drugs such as griseofulvin, danazol, and phenytoin, mainly due to their high thermodynamic driving force to crystallize. Guzman and coworkers introduced the concept of combining a rapidly dissolving and supersaturating “spring” excipient with a crystallization-inhibiting “parachute” to achieve and maintain high drug supersaturation. This concept can be realized in practice by exploiting the complimentary properties of individual components in a strategically designed polymer blend. Polymer blends are utilized in many advanced technologies including tough plastics, organic solar cells, gene delivery, and membrane separation. Although some effort has been directed at using polymer blends for drug delivery, the mechanisms that govern interactions between the active ingredients and excipients, and the ultimate effects on release and delivery, remain unclear. For example, Kalivoda and coworkers have observed both super-additive and simple averaging effects on dissolution enhancement using the same polymeric carriers for different drugs. These
studies suggest that polymer excipients must engage active ingredients through non-covalent interactions that inhibit nucleation and growth of crystals, both in the solid state and during hydration and release.\textsuperscript{158,159}

Micelle-forming polymers and surfactants have been widely studied as delivery vehicles to encapsulate hydrophobic drugs in the micelle core, with the hydrophilic corona serving as a stabilizing interface between the core and the aqueous medium.\textsuperscript{66,160,161} External stimuli-responsive mechanisms that can be triggered by changes in pH,\textsuperscript{68} temperature,\textsuperscript{69} or light\textsuperscript{70} are required for these micelle vehicles to release the stored drug. However, drug molecules trapped in a micelle core often suffer from low membrane permeability,\textsuperscript{162} resulting in poor drug bioavailability.\textsuperscript{163}

In this chapter, we take a different approach. By using blends of HPMCAS and a micelle forming end-functional homopolymer, the crucial difference is that the drug is stabilized in solution by partitioning into the corona of the micelle, rather than the core. It has been shown that hydrophilic homopolymers synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization using an alkyl chain transfer agent (CTA) can self-assemble into micelles in aqueous solution.\textsuperscript{120} The micelle properties (e.g., size and corona density) can be tuned by balancing the hydrophobic CTA chain length and the molecular weight of the hydrophilic portion of the molecule. We have exploited this behavior through water-soluble poly(\textit{N}-isopropylacrylamide) (PNIPAm) homopolymers containing a dodecyl end group, denoted as C\textsubscript{12}-PNIPAm. This compound self-assembles into spherical, star-like micelles when dissolved in phosphate-buffered saline (PBS, pH=6.5), resulting in a hydrated corona that can sequester phenytoin through preferential interactions with the NIPAm repeat units. Ternary blends of C\textsubscript{12}-PNIPAm, the commercially available excipient HPMCAS, and phenytoin were spray dried into solid dispersion particles, which immobilize the drug in an amorphous form. Subsequent hydration resulted in extraordinary levels of persistent drug supersaturation at specific
blend compositions. The interactions of the drug with the micelle corona are confirmed and quantified by NOESY and DOSY, and the suppression of both crystal nucleation and growth is revealed by cryo-TEM and optical microscopy. This work demonstrates an exciting new concept for optimizing polymer-based excipients. Physical entainment, immobilization and rapid dissolution of drug afforded by HPMCAS is combined synergistically with the crystallization-inhibiting ability of self-assembled C12-PNIPAm micelles, resulting in a polymer blend that delivers prolonged supersaturation of the rapidly crystallizing model drug phenytoin. The basic principles exposed by this study offer an attractive new platform for the development of enhanced excipients for oral drug delivery.

3.2 Experimental Section

Details of the instruments used in this chapter and most of the experimental procedures were outlined in Chapter 2. This section summarizes the sample preparation, experimental details, and instrument processing parameters that are specific to this chapter.

Materials.

Chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted: N-isopropylacrylamide (NIPAm, ≥ 99%), 2,2-azobis(2-methylpropionitrile) (AIBN, 98%), toluene (anhydrous, 99.8%), hexanes (99.9%, Fisher), methanol (HPLC grade, 99.9%), acetonitrile (HPLC grade, 99.9%), water (HPLC grade), tetrahydrofuran (THF, ≥ 99.9%), phenytoin (PTN, ≥ 99%), dimethyl sulfoxide-d₆ (DMSO-d₆, 99.9 atom% D, Cambridge Isotope Laboratories, Inc.), deuterium oxide (D₂O, 99.96 atom% D, Cambridge Isotope Laboratories, Inc.), chloroform-d (CDCl₃, 99.8 atom% D + 0.05% V/V TMS, Cambridge Isotope Laboratories, Inc.), 3-(trimethylsilyl)propionic-2,2,3,3-d₄
acid sodium salt (TSP, 98 atom% D). Hydroxypropyl methylcellulose acetate succinate (HPMCAS) (AFFINISOL, $M_n = 60$ kg/mol, $D = 2.4$, 10 wt% acetate, 11 wt% succinate) was supplied by the Dow Chemical Company and its general structure is shown in Figure 3.18d. Phosphate-buffered saline (PBS, pH=6.5) used for the dissolution studies contained 82 mM sodium chloride ($\geq 99\%$), 20 mM sodium phosphate dibasic heptahydrate ($99.4\%$, Mallinckrodt Chemicals), and 47 mM potassium phosphate monobasic ($\geq 99\%$, J.T. Baker). Fasted simulated intestinal fluid powder (FaSSIF) was purchased from Biorelevant, UK. The RAFT chain transfer agent (CTA) ($C_{12}$), $S$-1-dodecyl-$S$-($\alpha,\alpha'$-dimethyl-$\alpha''$-acetic acid) trithiocarbonate, was synthesized according to the procedure described elsewhere.$^{164}$ A second CTA ($C_2$), 4-cyano-4-[(ethylsulfanylthiocarbonyl) sulfanyl] pentanoic acid, was used as received (Strem Chemicals).

**Polymer Synthesis and Characterization**

Poly($N$-isopropylacrylamide) (PNIPAm) homopolymers were synthesized by RAFT polymerization according to the protocol shown in Figure 3.1, with number average molecular weight ($M_n$) and dispersity ($D$) summarized in Table 3.1. The polymers are designated as $C_2$-PNIPAm-7, $C_{12}$-PNIPAm-7, $C_{12}$-PNIPAm-30, and $C_{12}$-PNIPAm-98, with the prefix denoting the type of chain end and the suffix denoting $M_n$ in kg/mol.

**Table 3.1. Molecular characteristics of PNIPAm**

<table>
<thead>
<tr>
<th></th>
<th>$M_n$ (g/mol)</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_2$-PNIPAm-7</td>
<td>$7.0 \times 10^3$</td>
<td>1.03</td>
</tr>
<tr>
<td>$C_{12}$-PNIPAm-7</td>
<td>$6.7 \times 10^3$</td>
<td>1.03</td>
</tr>
<tr>
<td>$C_{12}$-PNIPAm-30</td>
<td>$3.0 \times 10^4$</td>
<td>1.17</td>
</tr>
<tr>
<td>$C_{12}$-PNIPAm-98</td>
<td>$9.8 \times 10^4$</td>
<td>1.27</td>
</tr>
</tbody>
</table>
Figure 3.1. Scheme of the RAFT polymerization of poly(N-isopropylacrylamide) (C\textsubscript{12}-PNIPAm and C\textsubscript{2}-PNIPAm). Protons are labeled for peak assignment in 2D \textsuperscript{1}H NOESY spectra shown in Figure 3.18.

In a typical polymerization targeting C\textsubscript{12}-PNIPAm-30, 15.0 g (0.133 mol) of NIPAm, 0.161g of \textit{S}-1-dodecyl-\textit{S}-(\textit{\alpha,\alpha'}-dimethyl-\textit{\alpha''}-acetic acid) trithiocarbonate (CTA(C\textsubscript{12}), 0.442 mmol), 7.25 mg of recrystallized AIBN (0.0442 mmol), and 67.5 mL of toluene were mixed in a 500 mL round-bottom flask. Three freeze-pump-thaw cycles were performed on the reactants and the flask was then filled with argon. The reaction was allowed to run at 72 °C while stirring for 12 h. The polymer product was dissolved in THF and precipitated by dropwise addition to n-hexane followed by filtration; this procedure was repeated twice to remove residual CTA and other byproducts. The final product was dried at approximately 50 °C for 24 h in a vacuum oven.

Molecular weight distributions were assessed by size-exclusion chromatography (SEC) and the chemical structures were confirmed by \textsuperscript{1}H nuclear magnetic resonance (NMR) spectroscopy. Details of these instruments are outlined in Chapter 2. The literature refractive index increment (dn/dc) of 0.107 mL/g was used for PNIPAm in THF.\textsuperscript{132} \textsuperscript{1}H NMR spectroscopy experiments were performed at 25 °C on a Bruker Avance III HD (500 MHz) instrument (equipped with a 5 mm Prodigy TCI cryoprobe) in
CDCl₃. SEC traces and ′H NMR spectra for PNIPAm homopolymers are shown in Figure 3.2 and Figure 3.3, respectively.

**Figure 3.2.** SEC curves of PNIPAm in THF with different molecular weights synthesized by RAFT polymerization (intensity from the refractive index detector).

**Figure 3.3.** ′H NMR spectrum of C₂-PNIPAm-7 and C₁₂-PNIPAm-7 in deuterated chloroform (CDCl₃) synthesized by RAFT polymerization.
**In vitro Dissolution Assay**

All SDDs were spray dried by using THF as the solvent. All of the *in vitro* dissolution tests were carried out under non-sink conditions (i.e., SI < 0.1 in Equation 1.4) over a six-hour experimental period at both 22 and 37 °C and the experimental details are outlined in Chapter 2.

**Polarized Light Microscopy**

A negative control of crystalline phenytoin in PBS/FaSSIF solution was prepared by a solvent shifting method. Phenytoin was dissolved in DMSO at a concentration of 50 mg/mL. 2 μL of the phenytoin/DMSO solution was dissolved in 98 μL of PBS/FaSSIF solution (2% v/v solvent shifting) so that the drug concentration in PBS/FaSSIF solution was 1000 μg/mL. After 4 minutes, a 20 μL aliquot was pipetted onto a pre-cleaned glass microscope slide (3 × 1”, Gold Seal) and the solution droplet was immediately covered with a glass microscope coverslip (24 × 40 mm, Fisher) for imaging with the same conditions described in Chapter 2.

**Cryogenic Transmission Electron Microscopy (cryo-TEM)**

The morphology and size of the nanostructures in the dissolution media of the SDDs were imaged by cryo-TEM in PBS solution during dissolution at 37 °C, experimental details are outlined in Chapter 2.

**2D ^1^H NMR Sample Preparation**

A D$_2$O solution containing 500 μg/mL TSP was first prepared by direct dissolution. HPMCAS or PNIPAm was dissolved in the D$_2$O solution and well mixed to achieve the desired polymer concentration (from 50 to 1000 μg/mL). Phenytoin was dissolved in DMSO-d$_6$ at a concentration of 5 mg/mL and this solution was pipetted into the prepared HPMCAS/D$_2$O or PNIPAm/D$_2$O solution with 1% DMSO-d$_6$/D$_2$O (v/v), so that the final phenytoin concentration in D$_2$O was 50 μg/mL.
3.3 Results and Discussion

Micellization of PNIPAm

Hydrodynamic radius ($R_h$) distributions of the PNIPAm homopolymers are shown in Figure 3.4, and the corresponding intensity-averaged $R_h$ values and dispersities ($\mu_2/\Gamma^2$) are summarized in Table 3.2.

Figure 3.4. Hydrodynamic radius distributions of PNIPAm in PBS solution (pH = 6.5, $T = 22 \, ^\circ C$) at the 90° scattering angle.
Table 3.2. Hydrodynamic radius and dispersity of PNIPAm in PBS solution (pH=6.5)

<table>
<thead>
<tr>
<th></th>
<th>C2-PNIPAm-7a</th>
<th>C12-PNIPAm-7a</th>
<th>C12-PNIPAm-30a</th>
<th>C12-PNIPAm-98a</th>
<th>C12-PNIPAm-98b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_h$ (nm)</td>
<td>$R_h$ (nm)</td>
<td>$R_h$ (nm)</td>
<td>$R_h$ (nm)</td>
<td>$R_h$ (nm)</td>
</tr>
<tr>
<td>1 mg/mL</td>
<td>7.8</td>
<td>12.0</td>
<td>16.6</td>
<td>24.5</td>
<td>7.9</td>
</tr>
<tr>
<td>5 mg/mL</td>
<td>0.12</td>
<td>0.14</td>
<td>0.30</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>9 mg/mL</td>
<td>0.09</td>
<td>0.19</td>
<td>0.38</td>
<td>8.6</td>
<td></td>
</tr>
</tbody>
</table>

a Fit to second-order cumulant expansion
b Fit to double exponential decay function

Note: Scattering intensity was too low (comparable to pure solvent) for C2-PNIPAm-7 at 1 mg/mL

The measured $R_h$ for C2-PNIPAm-7 is consistent with literature values for a single flexible PNIPAm chain in water with this molecular weight. With a longer RAFT end group, C12-PNIPAm-7 and C12-PNIPAm-30 self-assembled into micelles in PBS solution, consistent with previous reports that a dodecyl group is sufficient to induce assembly in aqueous media. The corresponding mean $R_h$ and dispersity ($\mu_2/\Gamma^2$) were calculated by fitting to a second-order cumulant expansion (Equation 2.2). At the highest molecular weight, C12-PNIPAm-98, equilibration between micelles and free chains is evidenced by a bimodal distribution. For C12-PNIPAm-98, the normalized autocorrelation functions ($g^{(2)}$) of the scattered light intensity at scattering angles from 60° to 120° in 15° increments were fitted to both a second-order cumulant model (Equation 2.2 in Chapter 2) and a double exponential decay model (Equation 3.1).

$$g^{(2)}(\tau) = B + (A_1 e^{-2\Gamma_1 \tau} + A_2 e^{-2\Gamma_2 \tau})^2$$  \hspace{1cm} (3.1)
where $\Gamma_1$ and $\Gamma_2$ are the decay rates of the two modes, $\tau$ is the delay time, $A_1$ and $A_2$ are the scattering intensity-weighted prefactors for the two decays and $B$ is the baseline. As shown in Figure 3.5, the residuals at all angles from the double exponential decay fit are smaller and more random compared to the ones from the second-order cumulant fit. Therefore, the double exponential decay model provided a better description of the data for C$_{12}$-PNIPAm-98. The peaks corresponding to micelles ($R_{h1}$) and free chains ($R_{h2}$) were obtained from the biexponential decay model fit.

![Figure 3.5](image)

**Figure 3.5.** Examples of autocorrelation function residuals for DLS data of 9 mg/mL C$_{12}$-PNIPAm-98 in PBS solution by using either (a) a second-order cumulant expansion fit or (b) a biexponential decay fit.

The decay rates ($\Gamma_1$ and $\Gamma_2$) vs. the square of the scattering vector ($q^2$) from the double exponential decay fits are shown in Figure 3.6. Linear regression yielded a slope of 7.1 x 10$^{-12}$ m$^2$/s and an intercept of 115 s$^{-1}$ for $R_{h1}$, and a slope of 2.6 x 10$^{-12}$ m$^2$/s and an intercept of 245 s$^{-1}$ for the $R_{h2}$ fit. The linear dependence of the decay rates on $q^2$ and the low values of the intercepts confirm that both relaxation processes are diffusive.
Representative SLS data of the PNIPAm homopolymers are plotted in the Debye-Zimm format (Figure 3.7) and the calculated micelle molecular weight ($M_{\text{agg}}$), aggregation number ($N_{\text{agg}}$) and second virial coefficient ($A_2$) are summarized in Table 3.3. C$_2$-PNIPAm-7 had an aggregation number of 1, consistent with individual flexible coils in the PBS solution. All the C$_{12}$-PNIPAm samples exhibited aggregation numbers greater than 1, consistent with micelle formation. The micelle corona density ($\rho_{\text{corona}}$) was estimated according to Equations 3.2-3 by assuming that the core density ($\rho$) equals that of the bulk CTA (C$_{12}$, $\rho = 1.082 \text{ g/cm}^3$):

$$\frac{4}{3} \pi R_c^3 \rho N_{\text{Av}} = N_{\text{agg}} M_{\text{end}}$$  \hspace{1cm} (3.2)
\[ \rho_{\text{corona}} = \frac{M_{\text{agg}}}{N_{\text{av}} \left( \frac{4}{3} \pi R_h^3 - \frac{4}{3} \pi R_c^3 \right)} \]  

(3.3)

where \( R_c \) is the core radius, \( N_{\text{av}} \) is Avogadro’s number, \( M_{\text{end}} \) is the molecular weight of the chain end (\( C_{12} \)), \( N_{\text{agg}} \) is the aggregation number, \( M_{\text{agg}} \) is the aggregate molecular weight, and \( R_h \) is the micelle hydrodynamic radius (obtained by fitting to a second-order cumulant model). Therefore, the average density of polymer in the micelle corona (\( \rho_{\text{corona}} \)) was estimated based on \( N_{\text{agg}} \) and \( R_h \) to be 136 ± 29, 46 ± 9, and 23 ± 4 mg/mL for \( C_{12}-\text{PNIPAm-7} \), \( C_{12}-\text{PNIPAm-30} \), and \( C_{12}-\text{PNIPAm-98} \), respectively. At a given overall polymer concentration, the number density of PNIPAm micelles is inversely proportional to \( M_{\text{agg}} \).

![Graph](image)

**Figure 3.7.** SLS data of PNIPAm in PBS solution (pH = 6.5, \( T = 22 \text{ °C} \)) at the 90° scattering angle.
Table 3.3. Summary of the SLS results

<table>
<thead>
<tr>
<th></th>
<th>$M_{agg}$ (g/mol)</th>
<th>$N_{agg}$</th>
<th>$A_2$ (mol cm$^3$/g$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_2$-PNIPAm-7</td>
<td>$7.3 \times 10^3$</td>
<td>1</td>
<td>$2.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>C$_{12}$-PNIPAm-7</td>
<td>$1.6 \times 10^5$</td>
<td>25</td>
<td>$1.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>C$_{12}$-PNIPAm-30</td>
<td>$2.3 \times 10^5$</td>
<td>7.7</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>C$_{12}$-PNIPAm-98</td>
<td>$3.4 \times 10^5$</td>
<td>3.5</td>
<td>$1.3 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Synergistic Effect of Polymer Blend on Drug Dissolution

Phenytoin, a BCS Class II compound, was selected as the model drug for this study because it has a very strong thermodynamic driving force to crystallize at low temperatures,$^{165}$ as predicted by the Hoffman equation.$^{166}$ To evaluate the efficacy of using polymer blends as excipients, we prepared SDDs at a constant phenytoin loading of 10 wt% and varying weight fractions of HPMCAS and C$_{12}$-PNIPAm-30. Representative SEM images of the SDDs are shown in Figure 3.8. A comparison of the three-component (e.g., 70-20) and two-component (e.g., 90-0 or 0-90) images revealed no obvious difference in the bulk morphology of the SDDs (i.e., they are all “raisin-like” collapsed spheres, Figure 3.8). All loaded phenytoin remained amorphous in the SDDs as demonstrated by featureless diffraction patterns under wide angle X-ray scattering (lab-scale WAXS, Figure 3.9).
Figure 3.8. Representative SEM images of crystalline phenytoin and various SDDs at a constant drug loading of 10 wt%. Scale bars equal 5 μm. The two values of the labels represent the weight percentages of HPMCAS and C_{12}-PNIPAm-30, respectively.

Figure 3.9. Wide angle X-ray scattering patterns of all SDDs at a constant phenytoin loading of 10 wt%. θ is the Bragg angle. The two values in the legend represent the weight percentages of HPMCAS and C_{12}-PNIPAm-30, respectively.
To assess phenytoin release and maintenance of supersaturation with the prepared SDDs, the apparent phenytoin concentration was measured using the *in vitro* microcentrifuge dissolution test. All dissolution tests were carried out under non-sink conditions in PBS/FaSSIF solution (pH=6.5) for six h, with a total phenytoin concentration of 1000 μg/mL, which was approximately 21 times higher than the measured equilibrium solubility of the crystalline form ($C_{\text{crystalline phenytoin}} = 48 \pm 1 \mu g/mL$ in PBS/FaSSIF solution at 37 °C). Phenytoin concentrations in the supernatant, measured by reverse-phase high-performance liquid chromatography (HPLC), are shown in Figure 3.10a as a function of time for various SDDs. The enhancement factor is defined as the ratio of the area under the dissolution concentration–time curve (AUC) of the SDD to the AUC of the crystalline phenytoin over a period of six hours:

$$\text{Enhancement Factor} = \frac{\text{AUC}(360 \text{ min})_{\text{SDD}}}{\text{AUC}(360 \text{ min})_{\text{crystalline drug}}} \quad (3.4)$$

With pure HPMCAS as the excipient (*i.e.*, 90-0), relatively high supersaturation levels of phenytoin ($C_{\text{max}} = 907 \pm 18 \mu g/mL$) were achieved initially. This high level, however, was not maintained as phenytoin crystallized and precipitated out of solution after 20 min (Figure 3.10 a). When using just C$_{12}$-PNIPAm-30 as the excipient (*i.e.*, 0-90), phenytoin exhibited no burst release and maintained an approximately constant, relatively low supersaturation level above the measured equilibrium concentration. A striking synergistic effect was observed with HPMCAS/C$_{12}$-PNIPAm-30 binary blend excipients, with the most dramatic result realized in the SDD 70-20 system. This formulation achieved relatively high phenytoin release and sustained a remarkable level of drug supersaturation throughout the experimental period ($C_{360 \text{ min}} = 840 \pm 50 \mu g/mL$), delivering a much better dissolution performance than either individual excipient (*i.e.*, 90-0 and 0-90).
90-0 and 0-90). As shown in Figure 3.10b, the 70-20 SDD provided an approximately 3-fold increase in the enhancement factor compared to 90-0. Similar synergistic effects were also obtained at 22 °C (see Figure 3.11). For all three-component SDDs shown in Figure 3.10a, the initial level of phenytoin supersaturation increased with increasing ratio of HPMCAS to PNIPAm. As the PNIPAm composition increased, the rate of drug concentration decline decreased. These observations support the hypothesis that HPMCAS serves as the main carrier, or “spring”, and facilitates rapid drug dissolution, while PNIPAm acts as a crystallization inhibitor, or “parachute”, prolonging drug supersaturation by inhibiting nucleation and/or crystal growth.

Figure 3.10. (a) Dissolution concentration–time curves for SDDs at 10 wt% drug loading at 37 °C, and (b) the corresponding enhancement factors (based on $C_{\text{crystalline phenytoin}} = 48 \pm 1 \mu\text{g/mL}$ in PBS/FaSSIF solution). The two values in the legend of (a) and labels of (b) represent the weight percentages of HPMCAS and C$_{12}$-PNIPAm-30, respectively.
Figure 3.11. Dissolution concentration–time curves for SDDs at 10 wt% drug loading at 22 °C (C\text{crystalline phenytoin} = 27 \pm 1 \mu g/mL in the PBS/FaSSIF solution at 22 °C). The two values in the legend of represent the weight percentages of HPMCAS and C\textsubscript{12}-PNIPAm-30, respectively.

Effect of PNIPAm Molecular Weight on Drug Dissolution

The influence of PNIPAm molecular weight on phenytoin dissolution was evaluated at three representative SDD compositions. With PNIPAm alone (\textit{i.e.}, 0-90), the dissolution performance was independent of molecular weight or chain end type at 37 °C over the molecular weight range of this study (\textit{i.e.}, \(M_n = 7 – 98\) kg/mol), as shown in Figure 3.12c. We hypothesize that the advantageous inhibition of nucleation and crystal growth was largely inoperative since the SDDs failed to achieve a high initial drug release over this molecular weight range for PNIPAm alone. However, for PNIPAm with even lower molecular weight, higher initial concentrations of phenytoin could be achieved and maintained with PNIPAm alone.\textsuperscript{13} Nevertheless, a significant PNIPAm molecular weight dependence was observed in the dissolution performance of the three-component SDDs (\textit{i.e.}, 70-20 and 80-10). As shown in Figures 3.12a and b, the SDDs containing the lower molecular weight C\textsubscript{12}-PNIPAm showed superior dissolution profiles.
Furthermore, the micelle-forming C₁₂-PNIPAm-7 also delivered a higher phenytoin supersaturation than free chains of C₂-PNIPAm-7 in both cases (i.e., 70-20 and 80-10). We attribute this important result to the micelle corona concentration, which will be explored in the section on drug-polymer interactions. Notably, the 70-20 SDD using C₁₂-PNIPAm-7 achieved approximately full release and maintenance of phenytoin throughout the six-hour experimental time (C₃₆₀₉₉₉nin = 923 ± 22 µg/mL). Similar molecular weight and chain end dependences were observed when performing the dissolution tests at 22 °C (see Figure 3.13).
Figure 3.12. Dissolution concentration−time curves for the SDDs of (a) 70-20, (b) 80-10, and (c) 0-90 HPMCAS-PNIPAm at 37 °C with different PNIPAm molecular weight and end groups.
Figure 3.13. Dissolution concentration–time curves for the SDDs of (a) 70-20, (b) 80-10, and (c) 0-90 at 22 °C with different PNIPAMs. The two values in the legend represent the weight percentages of HPMCAS and PNIPAm, respectively.
Drug Nucleation and Crystal Growth

To directly visualize and quantify phenytoin nucleation and crystal growth, we took polarized light micrographs of the dissolution media during the *in vitro* microcentrifuge dissolution test at 37 °C using HPMCAS/C12-PNIPAm-30 blends prepared at three compositions: 70-20, 80-10, and 90-0. As seen in Figure 3.14a, nucleation and growth of phenytoin crystals occurred with all three excipients. All crystals grown in the presence of the three polymer excipients were much smaller than the ones observed in a supersaturated phenytoin solution \((c = 1000 \, \mu g/mL)\) prepared by the solvent shift method (Figure 3.15). For SDD 70-20, both the total number of crystals and the average crystal radius were considerably smaller than those obtained from SDD 90-0 at equal time increments. SDD 80-10 nucleated a greater number of crystals than SDD 70-20 (but fewer than SDD 90-0) (Figure 3.14b), and grew approximately at the same rate as with SDD 70-20 (Figure 3.14c). Consistent with the dissolution tests, both the total number and the average radii of the crystals were smaller under PLM for polymer blend compositions that showed higher drug supersaturation during dissolution. Assuming each crystal appearing in the micrographs grew from an individual nucleus, the total crystal count over time provides a direct measure of the nucleation inhibition effect (neglecting nuclei that are smaller than the detection limit of PLM).\(^{167}\) On the other hand, the plot of average crystal radius versus time reveals how well the polymers inhibit crystal growth. We conclude from these experiments that PNIPAm micelles can inhibit both crystal nucleation and growth when used in appropriate combination with HPMCAS.
Figure 3.14. (a) Polarized light micrographs taken during dissolution in PBS/FaSSIF solution at 37 °C for the SDDs of 70-20, 80-10, and 90-0 (HPMCAS and C12-PNIPAm-30). Scale bars equal to 100 μm. Plots of (b) crystal count number per image and (c) crystal radius as a function of dissolution time.

Micrographs obtained from other compositions that exhibited lower drug supersaturation during dissolution revealed significantly larger phenytoin crystals and aggregates (Figure 3.15). In the absence of any polymer excipients, crystalline phenytoin precipitated out of the PBS/FaSSIF solution quickly and exhibited a long needle-like
morphology under polarized light (Figure 3.15b). As shown in Figure 3.15a, for SDDs that achieved lower supersaturation levels in dissolution tests (Figure 3.10), we observed aggregates of phenytoin crystals with much larger sizes than the ones shown in the Figure 3.14.

![Figure 3.15](image)

**Figure 3.15.** Polarized light micrographs of (a) three representative SDDs taken during dissolution in PBS/FaSSIF solution at 37 °C (b) crystalline phenytoin in PBS/FaSSIF solution. Scale bars equal 100 μm. The two values of the labels represent the weight percentages HPMCAS and C12-PNIPAm-30, respectively. Aggregates of phenytoin crystals were observed (indicated by arrows).

We employed cryogenic transmission electron microscopy (cryo-TEM) to visualize nanostructures in the dissolution media that were smaller than the spatial resolution of the
PLM technique (~250 nm). Representative cryo-TEM images of SDD 70-20 obtained from the HPMCAS/C_{12}-PNIPAm-7 blend during dissolution are shown in Figure 3.16. We hypothesize that the nanostructures evident in these images are phenytoin crystals or phenytoin-rich regions; no structures were observed in solutions of either HPMCAS or PNIPAm in the absence of phenytoin. Assuming that the nanostructures are spherical, analysis of the TEM images yields radii of 9 ± 3 at 10 min, 10 ± 4 at 90 min, and 13 ± 6 nm at 360 min during the dissolution test. Additional images at different time points are shown in Figure 3.17, and the morphologies and sizes of the observed nanostructures were similar compared to the ones shown in Figure 3.16. The cryo-TEM images further confirm that adding PNIPAm dramatically prolongs phenytoin supersaturation by retarding crystal growth. It has been reported that drug nucleation and crystal growth can be suppressed by drug-polymer interactions such as hydrogen bonding\textsuperscript{21,51,168} and hydrophobic interactions,\textsuperscript{169} which interfere with the crystal growth mechanism,\textsuperscript{165} as discussed in the following section.

![Figure 3.16. Representative cryo-TEM images of the SDDs of 70-20 (HPMCAS and C_{12}-PNIPAm-7) at (a) 10, (b) 90, and (c) 360 minutes during dissolution in PBS solution at 37 °C. Scale bars equal to 200 nm.](image)
Figure 3.17. Representative cryo-TEM images of the SDDs of 70-20 (HPMCAS and C\textsubscript{12}-PNIPAm-7) at (a) 40, (b) 20, (c) 90 and (d) 180 minutes during dissolution in PBS solution at 37 °C.

**Drug-Polymer Interactions**

We used NOESY to investigate how molecularly dissolved phenytoin (50 μg/mL) interacts with different polymers in solution. As shown in Figure 3.18a, the cross-peaks between the phenyl group of phenytoin around 7.4–7.5 ppm and the isopropyl group of the C\textsubscript{12}-PNIPAm-7 around 1.1–1.2 ppm indicate that the protons from these two groups are within a spatial proximity of approximately 5 Å.\textsuperscript{170} Similar results were obtained with C\textsubscript{12}-PNIPAm-30 and C\textsubscript{12}-PNIPAm-98 (see Figures 3.19 and 3.20, respectively). The isopropyl group peak of the PNIPAm repeat unit is clearly observed in the 1D spectrum sliced at the phenytoin phenyl group peak from the NOESY spectrum of phenytoin and C\textsubscript{12}-PNIPAm-30 (Figure 3.19), which indicates the existence of polymer-drug
intermolecular interactions in solution. The relative cross-peak intensity increases with mixing time (Figure 3.19), consistent with the absence of spin diffusion during the mixing time of the experiment.\textsuperscript{110} We infer that PNIPAm preferentially associates with phenytoin through hydrophobic interactions between the phenyl group on the drug and the isopropyl group on the polymer. In contrast, no cross-peaks were observed between phenytoin and C\textsubscript{2}-PNIPAm-7, as shown in Figure 3.18b. Qualitatively, this indicates that the intermolecular interactions between phenytoin and C\textsubscript{12}-PNIPAm-7 are stronger than between phenytoin and C\textsubscript{2}-PNIPAm-7 at equal phenytoin and polymer concentrations. In addition, there were no cross-peaks observed between phenytoin and HPMCAS at a much higher polymer concentration (1000 μg/mL, Figure 3.18c), indicative of much weaker interactions between these compounds.
Figure 3.18. 2D $^1$H NOESY spectra between 50 μg/mL phenytoin and (a) 50 μg/mL C$_{12}$-PNIPAm-7, (b) 50 μg/mL C$_2$-PNIPAm-7, and (c) 1000 μg/mL HPMCAS in deuterium oxide with a mixing time of 1.5 s. The cross-peaks in (a) between the peaks around 7.4–7.5 ppm of phenytoin and the isopropyl peaks around 1.1–1.2 ppm of C$_{12}$-PNIPAm-7 indicate polymer-drug intermolecular interactions in solution. (d) Molecular structures of phenytoin (PTN), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and poly(N-isopropylacrylamide) (PNIPAm). Protons are labeled for peak assignment in the 2D $^1$H NOESY spectra.
Figure 3.19. (a) 2D $^1$H NOESY spectrum between 50 μg/mL phenytoin and 50 μg/mL C$_{12}$-PNIPAm-30, (b) 1D spectrum sliced at 7.48 ppm from the 2D $^1$H NOESY spectrum of 50 μg/mL phenytoin and 50 μg/mL C$_{12}$-PNIPAm-30 in deuterium oxide. (c) Relative NOESY peak intensity (ratio of the cross peak intensity between protons a and c to the diagonal peak intensity at 7.48 ppm) as a function of mixing time.
Figure 3.20. 2D $^1$H NOESY spectrum between 50 μg/mL phenytoin and 50 μg/mL C$_{12}$-PNIPAm-98 in deuterium oxide.

The reduced diffusion coefficients ($D/D_0$) of phenytoin and trimethylsilyl propionic acid sodium salt (TSP) in polymer/D$_2$O solutions measured by DOSY are plotted in Figure 3.21 as a function of polymer concentration, where $D/D_0 = 1$ in the absence of polymer. Phenytoin and TSP concentrations were held at 50 and 500 μg/mL, respectively. The diffusion coefficient of TSP is independent of the polymer type, concentration or molecular weight, indicating that all DOSY experiments were performed in the dilute polymer regime.$^{100}$ Thus, changes in the macroscopic solution viscosity with polymer concentrations should not affect the diffusion of the small molecule tracers.$^{113}$ Nevertheless, the diffusion coefficient of phenytoin decreased with increasing C$_{12}$-
PNIPAm concentration, most dramatically with C\textsubscript{12}-PNIPAm-7, followed by C\textsubscript{12}-PNIPAm-30, and to the least extent with C\textsubscript{12}-PNIPAm-98. Within experimental error, the diffusion of phenytoin was unaffected by C\textsubscript{2}-PNIPAm-7, and HPMCAS had only a modest influence. These results provide strong support for the hypothesis that the corona of micelles formed from low molecular weight C\textsubscript{12}-PNIPAm most effectively sequester phenytoin in aqueous solution. We can model diffusion of phenytoin in the presence of PNIPAm using a simple two-state approach involving free and “bound” drug, whose mobilities differ by at least an order of magnitude (see Figures 3.22 and 3.23).\textsuperscript{113} Assuming bound phenytoin has the same diffusion coefficient as the associated polymer or polymer micelle,

\[
\langle D \rangle = P_{\text{free}} D_{\text{PTN,0}} + P_{\text{bound}} D_{\text{polymer}}
\]

where \( \langle D \rangle \) is the measured time-weighted diffusion coefficient, \( D_{\text{PTN,0}} \) is the diffusion coefficient of free phenytoin, \( D_{\text{polymer}} \) is the diffusion coefficient of the polymer, \( P_{\text{free}} \) represents the fraction of free drug, and \( P_{\text{bound}} \) represents the fraction of “bound” drug (\( i.e., P_{\text{free}} + P_{\text{bound}} = 1 \)). An association constant (\( K_b \)) can be used to quantify the extent of drug-polymer interaction,

\[
K_b = \frac{[\text{PTN}]_{\text{bound}}}{[\text{PTN}]_{\text{free}}[\text{repeat unit}]}\]

where [PTN]\text{bound} is the molar concentration of the bound phenytoin, [PTN]\text{free} is the molar concentration of the free phenytoin, and [repeat unit] is the molar concentration of the PNIPAm repeat unit.\textsuperscript{113} Association constants for phenytoin with each of the
PNIPAm molecules, averaged over all concentrations (solid curves), are listed in Figure 3.21. The value of $K_b$ decreases systematically with increasing C$_{12}$-PNIPAm molecular weight, from C$_{12}$-PNIPAm-7 ($K_b = 95 \pm 4$) to C$_{12}$-PNIPAm-98 ($K_b = 16 \pm 7$). Even less association was obtained with C$_{2}$-PNIPAm-7 ($K_b = 8 \pm 3$). These results show definitively that micelle formation greatly enhances association of phenytoin to PNIPAm, and that the most efficient excipients are the smallest self-assembled structures.

**Figure 3.21.** Reduced diffusion coefficients ($D/D_0$) of phenytoin (PTN) and trimethylsilyl propionic acid sodium salt (TSP) measured by DOSY in deuterium oxide ($D_2O$) as a function of polymer concentration. PTN and TSP concentrations were held as 50 and 500 µg/mL, respectively. Solid lines represent a two-state model that accounts for the diffusion of free and bound phenytoin, from which the indicated association constants $K_b$ were determined.
Figure 3.22. Representative fittings for the diffusion coefficient of PTN (50 μg/mL), TSP (500 μg/mL), and C12-PNIPAm-30 (50 μg/mL) in D2O.

Figure 3.23. Diffusion coefficients of PNIPAm as a function of polymer concentration, fitted from DOSY experiments.
3.4 Discussion

A single pharmaceutical product such as a tablet or capsule often contains a suite of polymers as excipients, and therefore to inform future excipient design it is important to understand why a clear synergy from the HPMCAS-PNIPAm blend was achieved in this case. Ilevbare and coworkers have previously observed synergistic effects in stabilizing supersaturated concentrations of the model drug ritonavir in the presence of seed crystals by using binary polymer combinations including cellulose and acrylamide-based polymers. They attributed the stability to the formation of interpolymer complexes that interact favorably with the crystallizing drug molecules and/or individual drug-polymer interactions at different adsorption sites. In contrast, we hypothesize that in our system each polymer interacts with phenytoin independently, and there is clear experimental evidence from both NOESY and DOSY (Figure 3.18 and Figure 3.21) that C12-PNIPAm interacts more strongly with phenytoin than does HPMCAS. In addition, we observed no NOESY cross peaks between HPMCAS and PNIPAm (Figure 3.24). Hence, we hypothesize that the main role of HPMCAS is to facilitate rapid phenytoin dissolution; this is consistent with other reports where cellulose-based polymers such as HPMCAS were able to rapidly deliver (i.e., “burst release”) a variety of hydrophobic molecules, including phenytoin. On the other hand, our results demonstrate unequivocally that PNIPAm independently inhibits both crystal nucleation and crystal growth to substantially sustain phenytoin supersaturation, resulting in a dramatic synergistic effect when blended with HPMCAS.
The lower critical solution temperature (LCST) of NIPAm-based thermoresponsive polymers is often exploited to trigger the release of an encapsulated drug.\textsuperscript{52,71,173} It is important to emphasize that the role of temperature (and the LCST) in this study is different, because the drug molecules are loaded in the micelle corona. The dissolution performance of the SDDs was modestly better (\textit{i.e.}, higher phenytoin supersaturation and AUC), at a temperature (37 °C) slightly above the cloud points of the PNIPAm homopolymers (see Figures 3.25 and 3.26, the cloud point was defined as the first temperature in heating that had less than 80\% transmittance) than slightly below (22 °C). Nevertheless, the composition and chain-end dependence described in the previous section apply at both temperatures (see Figures 3.10-13). When water becomes a poorer solvent for C\textsubscript{12}-PNIPAm at temperatures near or slightly above its cloud point, we
speculate that the contraction of the micelle corona and the subsequent formation of a micelle-rich phase should provide a more concentrated, relatively hydrophobic environment for stronger drug-polymer interactions, resulting in an enhanced supersaturation maintenance at 37 °C.

Figure 3.25. Representative transmittance vs. temperature traces for 9 mg/mL of the indicated PNIPAm samples in PBS/FaSSIF solution (heating rate = 0.25 °C/min)

Figure 3.26. Cloud points determined by optical transmittance measurement of the indicated PNIPAm samples at various concentrations in PBS/FaSSIF solution.
In the solid state, phenytoin, HPMCAS and PNIPAm are presumably homogenously mixed upon spray drying from THF, because of the kinetic trapping inherent to this process.\textsuperscript{82} Although this assumption has not been proven, it is not essential to the subsequent dissolution performance as long as no drug crystallization occurs (Figure 3.9). Upon dissolution from the SDDs, we hypothesize that C\textsubscript{12}-PNIPAm homopolymers should self-assemble into stable micelles for experiments performed at 22 °C, or subsequently begin to aggregate into a micelle-rich phase in solution at 37 °C. The effect of the remnant RAFT CTA on micelle formation has been previously studied, both by our group\textsuperscript{119} and by others.\textsuperscript{120,175} Our results here show that while micelle formation is not essential to achieve synergy in performance when blended with HPMCAS, the micelles are certainly very helpful in enhancing the favorable drug-polymer interactions. Dalsin and coworkers showed that pre-aggregation of diblock copolymer PEP-PDMA micelles could increase the dissolution rate and supersaturation maintenance of probucol and phenytoin compared to its non-micellar counterpart PDMA.\textsuperscript{108} However, in their work, the dissolution performance of these PEP-PDMA micelles was inferior to that of HPMCAS alone, especially for the rapidly crystallizing drug phenytoin.\textsuperscript{108} In contrast, here the phenytoin dissolution and supersaturation maintenance was significantly enhanced by blending HPMCAS with micelle-forming C\textsubscript{12}-PNIPAm. In addition, the initial drug supersaturation level that a polymeric excipient can achieve often decreases with increasing drug loading,\textsuperscript{23,53} suggesting that the drug-polymer ratio plays an important role in solubilizing and stabilizing drug molecules in solution. Hence, an optimal blending composition (\textit{i.e.}, 70-20) was observed in this work, mostly because a sufficient amount of each polymer was required to accomplish its corresponding role as rapid dissolver and crystallization inhibitor.

Recent work by Indulkar and coworkers showed that liquid–liquid phase separation often occurs for poorly water soluble drugs when the drug concentration is above its
amorphous solubility. These colloidal drug-rich nanodroplets were proposed to act as a reservoir to replenish absorbed drug during the intestinal absorption process.

The amorphous drug solubility \( c \) can be estimated by the following equation:

\[
c = \gamma c_{eq} \exp \left( \frac{\Delta G}{RT} \right)
\]

(3.7)

where \( \gamma \) is the activity coefficient of amorphous drug saturated with water (typically ranging from 0.5 to 1 for different drugs), \( c_{eq} \) is the equilibrium concentration of the crystalline drug, \( \Delta G \) is the free energy difference between the amorphous and crystalline form, \( R \) is the universal gas constant, and \( T \) is the actual temperature. The free energy difference between the amorphous and crystalline drug (\( \Delta G \)) can further be calculated based on the Hoffman Equation:

\[
\Delta G = \frac{\Delta H_f (T_m - T)T}{T_m^2}
\]

(3.8)

where \( \Delta H_f \) is the enthalpy of fusion, \( T \) is the actual temperature, and \( T_m \) is the drug melting temperature. The \( \Delta H_f \) and \( T_m \) values for phenytoin have been reported by Wassvik, et al. to be 40 kJ/mol and 296 °C, respectively. Assuming \( \gamma \approx 1 \), the predicted amorphous solubility of phenytoin is about 2000 \( \mu \)g/mL at 37 °C based on the equilibrium concentration of the crystalline phenytoin measured in this work (i.e., 48 \( \mu \)g/mL). In this study, the target phenytoin concentration is below its estimated amorphous solubility, and therefore, our system is unlikely to undergo liquid–liquid phase separation prior to drug crystallization, especially given that phenytoin is known to
be a rapid crystallizer. Therefore, we propose that micelles can also serve as a reservoir to store the hydrophobic drug in the slightly hydrophobic corona region, and inhibit drug crystallization by reducing the amount of free drug in aqueous phase.

The experimental results presented in this work also provide conclusive evidence of the mechanism of drug sequestration in C\textsubscript{12}-PNIPAm self-assembled micelles. Comparison of the data obtained from C\textsubscript{2}-PNIPAm-7 and C\textsubscript{12}-PNIPAm-7, nearly identical molecules with the exception of the length of the hydrophobic tail, clearly establishes the importance of micellization in thwarting nucleation and growth of drug crystals using the two-component excipient blend strategy. Increasing the molecular weight of C\textsubscript{12}-PNIPAm results in a decrease in the average concentration of NIPAm repeat units in the volume of space defined by the corona chains, as illustrated in Figure 3.27. Assuming half of the phenytoin molecules present in solution are stored in the corona (since the highest value of $P_{\text{bound}}$ from Equation 3.5 in this work is approximately 0.5 based on DOSY results at the highest polymer concentration), there are approximately 150 phenytoin molecules per C\textsubscript{12}-PNIPAm-7 micelle when using the SDD with optimal composition (\textit{i.e.}, 70-20), suggesting that each molecule associates with about ten NIPAm repeat units in the corona, on average. The cryo-TEM images may actually capture the nanostructures of C\textsubscript{12}-PNIPAm micelles loaded with phenytoin molecules (Figure 3.16). Although there is as yet no direct evidence that the drug in the nanostructures is amorphous, it is clear, based on PLM observations, that this system certainly prevents large-scale crystallization (and therefore precipitation). We propose that the weak non-covalent interactions that stabilize amorphous phenytoin in the presence of PNIPAm are enhanced at higher polymer concentration. Hence, greater crowding of the corona chains at lower molecular weight affords a more effective environment for maintaining the drug molecules in a solubilized state. Such cumulative effect of the corona environment for drug stabilization is analogous to the partitioning
effect of a non-polar stationary phase in liquid chromatography.\textsuperscript{179} Thus, at constant overall loading with HPMCAS, low molecular weight C\textsubscript{12}-PNIPAm outperforms a higher molecular weight analogue. By storing and delivering the small drug molecules in the micelle corona, rather than the core, it may also prove possible to resolve the low membrane permeability issue\textsuperscript{162} that most micelle encapsulation vehicles suffer in oral drug delivery. Thus, this discovery has important implications for the design of excipients in general.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{micelle_corona.png}
\caption{Illustration of the micelle corona sequestration mechanism to store drug molecules.}
\end{figure}

3.5 Conclusions

By using mixtures of HPMCAS and PNIPAm, we demonstrate that a polymer blend system that combines a main carrier to facilitate rapid drug dissolution and a crystallization inhibitor to prolong the drug supersaturation level provides a simple yet powerful strategy to enhance the solution availability of poorly water-soluble drugs (e.g., phenytoin). Synergistic effects were observed during in vitro dissolution tests for spray-dried dispersions using HPMCAS-PNIPAm blends at constant phenytoin loading.
Polarized light and cryo-TEM micrographs of the dissolution media confirmed that adding PNIPAm suppressed both crystal nucleation and crystal growth, by sequestering the phenytoin via favorable phenytoin-PNIPAm interactions. These interactions were clearly revealed by NOESY, and their strength was quantified by DOSY.

Most importantly, these results showcase a novel strategy to inhibit drug nucleation and crystal growth by “storing” molecularly dissolved drug molecules in the coronas of self-assembled micelles. With the dodecyl RAFT CTA as the micelle core, C12-PNIPAm self-assembled into well-defined micelles that became smaller and denser with decreasing PNIPAm molecular weight. The PNIPAm coronas served as a pocket or reservoir to associate and stabilize hydrophobic phenytoin in aqueous media. The results revealed a remarkably strong correlation between the phenytoin-PNIPAm association constant ($K_b$) and polymer micelle corona density; $K_b$ increased with increasing micelle corona density when the molecular weight of C12-PNIPAm was reduced, resulting in a higher phenytoin supersaturation and maintenance during in vitro dissolution test when blended with HPMCAS in SDDs at equal phenytoin loading and blend compositions. This work opens up a new platform for oral therapeutic delivery vehicles by storing hydrophobic drug molecules in micelle coronas. This strategy, along with the general concept of using tailored polymer blends as excipients, can potentially be generalized and applied to deliver any hydrophobic small molecules by rational design of specific molecule-polymer interactions.
4. Micelle-forming Diblock Polymers


4.1 Introduction

As shown in the previous chapter, the choice of the polymer excipient(s) in an amorphous solid dispersion (ASD) determines the ultimate dissolution behavior of the drug in aqueous solution. A variety of polymer vehicles have been used to achieve rapid dissolution and supersaturation maintenance, yet there is not a single mechanism that can be universally applied to all drug candidates. Over recent decades, block polymers with core-corona micellar structures in aqueous solution have proven to be effective carriers for the delivery of hydrophobic drugs. The amphiphilic character of the block polymer micelles enables a high drug uptake in the hydrophobic inner core, surrounded and stabilized by the hydrophilic corona. A major challenge for such a core-loading strategy is the low permeability of the entrapped drug molecules through the gastrointestinal membrane, which is just as important as the aqueous solubility for enhanced bioavailability of orally administered drugs. Although numerous external stimuli-responsive mechanisms have been developed to trigger the controlled release of the entrapped drugs, these approaches cannot be easily realized in the human GI tract for oral drug delivery applications. To resolve this problem, recent works (including the approach used in Chapter 3) have shown that hydrophobic drug molecules can be sequestered in the micelle corona, rather than the core, for enhanced solubility and supersaturation maintenance. Analogous to the mechanism of reverse phase liquid chromatography, the micelle corona serves as a “reservoir” to stabilize a high drug
supersaturation by partitioning these hydrophobic drug molecules into a less hydrophilic polymer-rich phase.\textsuperscript{11,13}

In this chapter, we systematically investigate the effect of the solution state assembly on drug-polymer interactions and the subsequent \textit{in vitro} dissolution of the ASDs. Three poly(\(N\)-isopropylacrylamide-\textit{co-}\(N,N\)-dimethylacrylamide)-\textit{b}-polystyrene (PND-\textit{b}-PS-\(C_{12}\)) diblock polymers with an identical thermoresponsive PND corona block and a hydrophobic polystyrene core block of different molecular weights were synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization, along with two PND statistical copolymer controls with either dodecyl (\(C_{12}\)) or ethyl (\(C_{2}\)) chain ends (Figures 2.1-2). It has been shown that \(N\)-isopropylacrylamide (NIPAm)-based polymers, synthesized by RAFT polymerization using an alkyl chain transfer agent (CTA), self-assembled into micelles in aqueous solution with a \(C_{12}\) core, but maintained an individual random coil conformation with a \(C_{2}\) chain end.\textsuperscript{11,119,120} Thus, in this study, the properties of the micelles (i.e., size, corona density and thickness) were systematically investigated by varying the length of the hydrophobic polystyrene block. The solution-state properties of the statistical copolymers and diblock polymers in phosphate-buffered saline (PBS, pH = 6.5) were characterized by dynamic light scattering, static light scattering, small angle X-ray scattering, and cryogenic transmission electron microscopy.

Phenytoin (PTN), an anticonvulsant, and nilutamide (NID), a nonsteroidal antiandrogen, were selected as the model drugs for this study. Although both drugs suffer low aqueous solubility, PTN is not only more hydrophobic, but also a more rapid crystallizer, than NID.\textsuperscript{165,184} Drug-polymer intermolecular interactions in aqueous solution, with the polymers as either free chains or micelles, were investigated by 2D \(^1\)H nuclear Overhauser effect spectroscopy (NOESY), and their corresponding “binding” strength was further quantified by 2D \(^1\)H diffusion ordered spectroscopy (DOSY, also known as pulsed-field-gradient NMR). The polymer free chains or micelles were then
spray dried with the two model drugs, and the \textit{in vitro} dissolution of the ASDs was systematically studied as a function of drug type, drug loading, and solution-state assembly of the polymers by using either a selective or non-selective solvent prior to spray drying. The important influence of micelle assembly on drug-polymer interactions and subsequent \textit{in vitro} dissolution performance in the corona-loading strategy revealed by this study can lead to the rational design of polymer micelles as effective therapeutic formulations.

\section*{4.2 Experimental Section}

Details of the instruments used in this chapter and most of the experimental procedures were outlined in Chapter 2. This section summarizes the sample preparation, experimental details, and instrument processing parameters that are specific to this chapter.

\section*{Materials}

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted: phenytoin (PTN, \( \geq 99\% \)), nilutamide (NID), \( N\)-isopropylacrylamide (NIPAm, \( \geq 99\% \)), \( N,N\)-dimethylacrylamide (DMA, \( 99\% \)), styrene (S, \( \geq 99\% \)), 2,2-azobis(2-methylpropionitrile) (AIBN, \( 98\% \)), 1,4-dioxane (anhydrous, \( 99.8\% \)), tetrahydrofuran (THF, \( \geq 99.9\% \)), hexanes (99.9\% , Fisher), methanol (HPLC grade, 99.9\%), acetonitrile (HPLC grade, 99.9\%), water (HPLC grade), dimethyl sulfoxide-d\(_6\) (DMSO-d\(_6\), 99.9 atom\% D, Cambridge Isotope Laboratories, Inc.), deuterium oxide (D\(_2\)O, 99.9 atom\% D, Cambridge Isotope Laboratories, Inc.), chloroform-d (CDCl\(_3\), 99.8 atom\% D + 0.05\% v/v TMS, Cambridge Isotope Laboratories, Inc.), 3-(trimethylsilyl)propionic-2,2,3,3-d\(_4\) acid sodium salt (TSP, 98 atom\% D). Hydroxypropyl methylcellulose acetate succinate (HPMCAS) was provided by the Dow Chemical Company (AFFINISOL, \( M_n = \))
60 kg/mol, $D = 2.4$, 10 wt% acetate, 11 wt% succinate). The RAFT chain-transfer agent (CTA), $S$-1-dodecyl-$S$-($\alpha,\alpha'$-dimethyl-$\alpha''$-acetic acid) trithiocarbonate ($C_{12}$), was synthesized according to procedures adapted from the literature. Another CTA, 4-cyano-4-[(ethylsulfanylthiocarbonyl) sulfanyl] pentanoic acid ($C_2$), was purchased from Strem Chemicals, Inc. and used as received. Phosphate buffered saline (PBS, pH = 6.5) was prepared using Milli-Q water with 82 mM sodium chloride ($\geq 99.9\%$, Fisher), 20 mM sodium phosphate dibasic ($99.5\%$), and 47 mM potassium phosphate monobasic ($\geq 99\%$, J.T. Baker). Fasted simulated intestinal fluid powder (FaSSIF) was purchased from Biorelevant.

**Polymer Synthesis and Characterization**

Synthesis of poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-polystyrene (PND-b-PS-C12) is described in detail in Chapter 2. The compositions of NIPAm for PND$_{34}$-C$_2$ and PND$_{34}$-C$_{12}$ were both 67 mol%, based on $^1$H NMR analysis shown in Figure 2.5 in Chapter 2. The polystyrene compositions for PND-$b$-PS-C$_{12}$ diblock polymers were also calculated from $^1$H NMR spectra (Figure 2.5). SEC traces of the polymers are shown in Figure 2.4 in Chapter 2. Polystyrene molecular weight for the PND-$b$-PS-C$_{12}$ diblock polymers was determined based on the PND molecular weight from SEC and the polystyrene composition from $^1$H NMR. The polymer characteristics are also summarized in Table 2.1 in Chapter 2.

**Micelle Formation**

PND-$b$-PS-C$_{12}$ diblock polymer micelles were prepared using the cosolvent method. PND-$b$-PS-C$_{12}$ was dissolved in THF at 10 mg/mL. An equal volume of Milli-Q water was titrated dropwise into the THF solution while stirring. Typically, the transparent solution became translucent after addition of approximately 15% of the total volume of Milli-Q water, indicating the formation of micelles. The solution was transferred into a
dialysis bag and dialyzed against a large amount of DI water for 3 days to remove THF. The solution was then passed through a 0.45 μm filter to remove dust and residual large aggregates, after which the solution became transparent. Finally, the solution was lyophilized to produce micelles with frozen polystyrene cores in the solid state.

2D 1H NMR Sample Preparation

A stock solution of deuterated PBS solution consisting of 82 mM sodium chloride, 20 mM sodium phosphate dibasic, and 47 mM potassium phosphate monobasic in D2O was prepared. TSP was added directly to the solution at a concentration of 50 or 100 μg/mL for study of PTN or NID, respectively. PND statistical polymers were dissolved directly in the solution at various concentrations. Lyophilized PND-b-PS-C12 micelles, prepared by the cosolvent method, were dissolved in the solution to achieve equal PND concentrations as the PND statistical polymers. PTN or NID was then dissolved in DMSO-d6 at a concentration of 5 or 10 mg/mL, respectively. The DMSO-d6 solution was pipetted into the prepared polymer/D2O buffer mixture at 1% DMSO-d6/D2O (v/v), to achieve a final PTN or NID concentration in the deuterated PBS solution of 50 or 100 μg/mL for each study, respectively.

Diffusion Ordered Spectroscopy (DOSY)

For PTN, the “ledbpgp2s” pulse sequence was applied with a relaxation delay of 3 s and 32 scans in total. For NID, the “stebpgp1s19” pulse sequence (stimulated echoes using bipolar gradient with 1 spoil gradient for diffusion and using 3-9-19 pulse sequence with gradients for water suppression) was applied with a relaxation delay of 3 s and 128 scans in total.

Cryogenic Transmission Electron Microscopy (cryo-TEM)

Lyophilized diblock polymer micelles were dissolved in PBS solution at 37 °C for cryo-TEM imaging (details outlined in Chapter 2).
4.3 Results

Micellization of Polymer

The polymers investigated in this work all exhibited cloud points above body temperature at 40-41 °C (Figure 4.1), due to the copolymerization of the more hydrophilic monomer DMA (33 mol%) with NIPAm (67 mol%) (see Figure 2.5). The cloud points are defined as the first temperature on heating that had less than 80% transmittance.

![Figure 4.1](image)

Figure 4.1. Cloud points determined by optical transmittance measurement of the polymers at 10 mg/mL in PBS solution (heating rate = 0.25 °C/min). The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution.

Hydrodynamic radius distributions of the polymers in PBS solution (pH = 6.5, $T = 25$ °C) are shown in Figure 4.2. The corresponding mean hydrodynamic radius ($R_h$) and dispersity ($\mu_2/I^2$) at both 25 °C and 37 °C were calculated by fitting to a second-order cumulant model (Equation 2.2), and are summarized in Table 4.1. Representative
autocorrelation functions and plots of decay rate $\Gamma$ versus $q^2$ are shown in Figure 2.6. The two PND statistical copolymers with the same molecular weight but different RAFT end groups showed very different $R_h$ values upon direct dissolution in PBS solution, indicating that PND$_{34}$-C$_2$ and PND$_{34}$-C$_{12}$ exhibited free chain and micelle conformations in PBS solution, respectively. This result is consistent with previous studies in our group and by others, that the dodecyl RAFT end group is sufficiently hydrophobic to facilitate micellar self-assembly of NIPAm-based homopolymers in aqueous solution.$^{13,119,120}$ The diblock polymer micelles with frozen polystyrene cores (i.e., prepared by the cosolvent method) exhibited narrowly distributed hydrodynamic radii upon dissolution in PBS solution. At the same temperature, the mean $R_h$ value increased with increasing PS chain length for the diblock micelles, mostly due to the increased core size. As the temperature increased from 25 °C to 37 °C, values of both $R_h$ and dispersity ($\mu^2/\Gamma^2$) of the diblock micelles decreased. This is attributed to the contraction of the PND corona chains as the temperature approached the lower critical solution temperatures (LCSTs, 40-41 °C) of the corona blocks.
Figure 4.2. Hydrodynamic radius distributions at 10 mg/mL in PBS solution (pH = 6.5, $T = 25 \, ^\circ C$) at 90° scattering angle. The diblock solutions were prepared by the cosolvent method and lyophilized before dissolution.

Table 4.1. Mean hydrodynamic radius and dispersity of polymers in PBS solution (pH = 6.5)

<table>
<thead>
<tr>
<th></th>
<th>$T = 25 , ^\circ C$</th>
<th></th>
<th>$T = 37 , ^\circ C$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_h$ (nm)</td>
<td>$\mu_2/I^2$</td>
<td>$R_h$ (nm)</td>
</tr>
<tr>
<td>PND$_{34}$-C$_2$</td>
<td>4.9</td>
<td>0.19</td>
<td>5.7</td>
</tr>
<tr>
<td>PND$<em>{34}$-C$</em>{12}$</td>
<td>13.0</td>
<td>0.09</td>
<td>15.9</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>2$-C$</em>{12}$</td>
<td>20.2</td>
<td>0.09</td>
<td>19.7</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>9$-C$</em>{12}$</td>
<td>24.4</td>
<td>0.02</td>
<td>22.7</td>
</tr>
<tr>
<td>PND$<em>{34}$-b-PS$</em>{14}$-C$_{12}$</td>
<td>29.0</td>
<td>0.01</td>
<td>27.0</td>
</tr>
</tbody>
</table>

The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution.

SAXS measurements were conducted to determine the core radius ($R_c$) and aggregation number ($N_{agg}$) of the polymer micelles (summarized in Table 4.2). Figure 4.3
shows the SAXS patterns of all five polymers in 10 mg/mL PBS solution at 25 °C (solvent background was subtracted, see Figure 4.4).

Figure 4.3. Small-angle X-ray scattering (SAXS) data of the polymer samples at 10 mg/mL in PBS solution (pH = 6.5, T = 25 °C), with PBS solution scattering subtracted. The solid lines are best fits to the Pedersen model, except for PND$_{34}$-C$_2$ (fit to the Debye model) and PND$_{34}$-C$_{12}$ (fit to the Gaussian star polymer model). The profiles are shifted vertically by factors of 10 for clarity. The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution.
**Figure 4.4.** Small-angle X-ray scattering (SAXS) data of (a) PND$_{34}$-C$_{2}$ and (b) PND$_{34}$-C$_{12}$ at 10 mg/mL in PBS solution (pH = 6.5, $T = 25$ °C). The samples were prepared by direct dissolution. Solid lines are best fits to (a) the Debye function, and (b) a Gaussian star polymer model, respectively.

**Table 4.2.** Micelle characteristics based on fitting SAXS results at 25 °C

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_c$ (nm)</th>
<th>$N_{agg}$$^{a,b}$</th>
<th>$L_{corona}$$^c$ (nm)</th>
<th>$\rho_{corona}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND$<em>{34}$-C$</em>{12}$</td>
<td>-</td>
<td>12 ± 1</td>
<td>13.0</td>
<td>74 ± 13</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>2$-C$</em>{12}$</td>
<td>2.0 ± 0.1</td>
<td>11 ± 2</td>
<td>18.2</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>PND$_{34}$-b-PS$<em>9$-C$</em>{12}$</td>
<td>5.3 ± 0.1</td>
<td>45 ± 5</td>
<td>19.1</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>PND$<em>{34}$-b-PS$</em>{14}$-C$_{12}$</td>
<td>9.1 ± 0.2</td>
<td>139 ± 14</td>
<td>19.9</td>
<td>79 ± 16</td>
</tr>
</tbody>
</table>

*a.* $N_{agg}$ = number of arms ($f$) of the Gaussian star polymer model fit for PND$_{34}$-C$_{12}$

*b.* $N_{agg}$ of the diblock polymers is estimated by assuming a dry core

*c.* $L_{corona} = R_h - R_c$

SAXS data of the polymers were fit to different models. Scattering intensity ($I$) is a function of the form factor [$P(q)$] and background ($B$, i.e., scattering intensity from PBS solution and capillary tube), as shown in Equation 4.1. An arbitrary prefactor (A) was used because the SAXS data were not reduced to an absolute intensity scale.

$$I = A \cdot P(q) + B \quad (4.1)$$
SAXS data of PND$_{34}$-C$_2$ free chains (Figure 4.4a) were fit to the Debye function,\textsuperscript{185} and the form factor is shown in the following equations:

$$P(q) = \frac{2}{x^2} \left( e^{-x} - 1 + x \right)$$ \hspace{1cm} (4.2)

$$x = q^2 R_g^2$$ \hspace{1cm} (4.3)

where $q$ is the scattering vector and $R_g$ is the radius of gyration. SAXS data of PND$_{34}$-C$_{12}$ micelles (Figure 4.4) were fit to the Gaussian star polymer model, described in detail elsewhere.\textsuperscript{186,187} The form factor $[P(q)]$ of the Gaussian star polymer model is shown in the following equations:

$$P(q) = \frac{2}{f v^4} \left[ v^2 - \left( 1 - e^{-v^2} \right) + \frac{f - 1}{2} \left( 1 - e^{-v^2} \right)^2 \right]$$ \hspace{1cm} (4.4)

$$v = \sqrt{\frac{f}{3f - 2}} q R_g$$ \hspace{1cm} (4.5)

where $q$ is the scattering vector, $f$ is the number of arms, and $R_g$ is the radius of gyration.

SAXS data of the diblock polymer micelles (Figure 4.5) were fit to the Pedersen model.\textsuperscript{188} The form factor $[P(q)]$ describes a micelle with a spherical core and Gaussian polymer chains attached to the surface, as shown in Equation 4.6,\textsuperscript{188}

$$P(q) = N_{agg}^2 \beta_s^2 F_s(q) + N_{agg} \beta_c^2 F_c(q) + 2 N_{agg}^2 \beta_s \beta_c S_{sc}(q) + N_{agg} (N_{agg} - 1) \beta_c^2 S_{cc}(q)$$ \hspace{1cm} (4.6)
where $N_{agg}$ is the micelle aggregation number, $\beta_s$ is the total access scattering length of the core block, and $\beta_c$ is the total access scattering length of the corona block. $F_s(q)$ describes the self-correlation of the spherical core, $F_c(q)$ describes the self-correlation of the Gaussian chains in the corona, $S_{sc}(q)$ is the interference cross term between the core and the corona chains, and $S_{cc}(q)$ is the interference term between the chains in the corona. Detailed functions for these terms were previously presented by Pedersen. The polystyrene core radius ($R_c$) for the micelle was obtained by fitting to Equation 4.6, and the resulting $R_c$ values are summarized in Table 4.2.

**Figure 4.5.** Small-angle X-ray scattering (SAXS) data of (a) PND$_{34}$-b-PS$_2$-C$_{12}$, (b) PND$_{34}$-b-PS$_9$-C$_{12}$, and (c) PND$_{34}$-b-PS$_{14}$-C$_{12}$ at 10 mg/mL in PBS solution (pH = 6.5, $T = 25 \, ^\circ C$). The samples were prepared by the cosolvent method and lyophilized before dissolution. Solid lines are best fits to the Pedersen model.
Static light scattering (SLS) confirmed the free chain conformation of PND\textsubscript{34}-C\textsubscript{2} in PBS solution \((N_{agg} = 1, \text{ see Table 4.3 and Figure 4.6}).\) The SAXS pattern of PND\textsubscript{34}-C\textsubscript{2} in PBS solution was fit to the Debye function (Equations 4.2-3),\textsuperscript{185} resulting in a radius of gyration \((R_g)\) value of 6.0 ± 0.4 nm. For the self-assembled PND\textsubscript{34}-C\textsubscript{12} micelles, the SAXS pattern was fit to a Gaussian star polymer model (Equations 4.4-5, the C\textsubscript{12} core size was negligible due to the enormous molecular weight difference between the core and corona).\textsuperscript{186} The aggregation number of the micelles was 12 ± 1, as estimated by the number of arms \((f)\) from the star polymer model fit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(M_{Agg}) (g/mol)</th>
<th>(N_{Agg})</th>
<th>(A_2) (mol cm(^2)/g(^2))</th>
<th>(R_g) (nm)</th>
<th>(R_c\textsuperscript{a}) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND\textsubscript{34}-C\textsubscript{2}</td>
<td>3.3 × 10(^4)</td>
<td>1 ± 0.02</td>
<td>3.7 × 10(^{-4})</td>
<td>20 ± 6</td>
<td>-</td>
</tr>
<tr>
<td>PND\textsubscript{34}-C\textsubscript{12}</td>
<td>3.1 × 10(^5)</td>
<td>9 ± 0.2</td>
<td>7.1 × 10(^{-5})</td>
<td>22 ± 5</td>
<td>1.0</td>
</tr>
<tr>
<td>PND\textsubscript{34}-b-PS\textsubscript{2}-C\textsubscript{12}</td>
<td>6.9 × 10(^5)</td>
<td>19 ± 1</td>
<td>-6.6 × 10(^{-4})</td>
<td>16 ± 10</td>
<td>2.4</td>
</tr>
<tr>
<td>PND\textsubscript{34}-b-PS\textsubscript{9}-C\textsubscript{12}</td>
<td>2.5 × 10(^6)</td>
<td>60 ± 2</td>
<td>-1.7 × 10(^{-5})</td>
<td>20 ± 10</td>
<td>5.8</td>
</tr>
<tr>
<td>PND\textsubscript{34}-b-PS\textsubscript{14}-C\textsubscript{12}</td>
<td>4.5 × 10(^6)</td>
<td>93 ± 1</td>
<td>-1.2 × 10(^{-5})</td>
<td>22 ± 2</td>
<td>8.0</td>
</tr>
</tbody>
</table>

\(a\). Core radii \((R_c)\) are estimated by assuming dry micelle cores.
Figure 4.6. Zimm plot of PND$_{34}$-C$_2$ in PBS buffer (pH = 6.5) at 27 °C.

Based on the fitting results shown in Table 4.2, the core radius of the micelle increased with increasing molecular weight of the polystyrene block. This trend, and the spherical shape of the polystyrene core, were further confirmed by cryogenic transmission electron microscopy (cryo-TEM) images of the diblock polymer micelles in PBS solution (Figure 4.7). In all cases, the frozen PS cores exhibited spherical morphologies. Based on the image analysis, core radii ($R_c$) are 3.5 ± 0.7, 6.0 ± 2.0, and 7.5 ± 2.1 nm for PND$_{34}$-b-PS$_2$-C$_{12}$, PND$_{34}$-b-PS$_9$-C$_{12}$, and PND$_{34}$-b-PS$_{14}$-C$_{12}$, respectively. Because the polystyrene core was kinetically frozen in aqueous solution at the experimental temperature, the micelle aggregation number was estimated by assuming a dry core. The micelle aggregation number ($N_{agg}$, upper bound) and corona density ($\rho_{corona}$) were calculated according to Equations 4.7 and 4.8, respectively:
\[
\frac{4}{3} \pi R^3_c \rho N_{av} = N_{agg} M_{n,PS}
\]  

(4.7)

\[
\rho_{\text{corona}} = \frac{N_{\text{agg}} M_{n,\text{PND}}}{N_{av} \left( \frac{4}{3} \pi R^3_h - \frac{4}{3} \pi R^3_c \right)}
\]  

(4.8)

where \( R_c \) is the core radius, \( \rho \) is the polystyrene core density, \( N_{av} \) is Avogadro’s number, \( M_{n,PS} \) is the molecular weight of polystyrene, \( M_{n,PND} \) is the molecular weight of PND, and \( R_h \) is hydrodynamic radius of the micelle.

The aggregation numbers of the diblock polymer micelles increased with increasing molecular weight of the polystyrene block, indicating that a larger number of chains can be incorporated into a core with a larger core size. The micelle core radius estimated from SAXS was consistent with the results obtained from both SLS and cryo-TEM within experimental uncertainty (Table 4.3 and Figures 4.8-10). Based on the DLS and SAXS
results, the corona thickness \((L_{\text{corona}} = R_h - R_c)\) and corona density \((\rho_{\text{corona}})\) of the micelles were calculated, and are summarized in Table 4.2. For the diblock micelles, the corona thickness increased with increasing molecular weight of the polystyrene block, suggesting that the corona chains were more stretched with a higher aggregation number. A more crowded corona environment (i.e., higher \(\rho_{\text{corona}}\)) was therefore achieved by increasing the length of the polystyrene block for these diblock micelles. The self-assembled PND\(_{34}\)-C\(_{12}\) micelle showed a smaller corona thickness compared to the diblock micelles, and therefore, its corona density was comparable to the diblock micelle with the longest polystyrene block (i.e., PND\(_{34}\)-b-PS\(_{14}\)-C\(_{12}\)). The correlation between the drug-polymer interaction and micelle corona density is discussed later.

\[\text{Figure 4.8.} \text{ Zimm plot of PND}_{34}\text{-}b\text{-PS}_{14}\text{-C}_{12} \text{ in PBS buffer (pH = 6.5) at 27 °C.}\]
Figure 4.9. Zimm plot of PND$_{34}$-b-PS$_9$-C$_{12}$ in PBS buffer (pH = 6.5) at 27 °C.

Figure 4.10. Zimm plot of PND$_{34}$-b-PS$_2$-C$_{12}$ in PBS buffer (pH = 6.5) at 27 °C.
Drug-Polymer Interactions

NOESY experiments were employed to explore drug-polymer interactions in deuterated PBS solution. PTN and NID were dissolved in deuterated PBS solution at 50 and 100 μg/mL, respectively. Cross-peak correlations of the aromatic group of PTN with both the isopropyl and methyl groups of PND<sub>34</sub>-b-PS<sub>14</sub>-C<sub>12</sub> were observed (Figure 4.11a), indicating that PTN molecules were in close spatial proximity to both NIPAm and DMA repeat units of the PND<sub>34</sub>-b-PS<sub>14</sub>-C<sub>12</sub> micelle corona. The 1D spectrum sliced at the PTN aromatic group peak (i.e., 7.48 ppm) from the 2D <sup>1</sup>H NOESY spectrum of PTN and PND<sub>34</sub>-b-PS<sub>14</sub>-C<sub>12</sub> confirmed these correlations, since both the isopropyl group from the NIPAm repeat unit and methyl groups from the DMA repeat unit were clearly observed in the 1D spectrum (Figure 4.12). This indicates that PTN molecules interacted with the micelle corona, rather than the core. At equal PTN and PND concentrations, no drug-polymer cross-peaks were observed between PTN and PND<sub>34</sub>-C<sub>2</sub> (Figure 4.13a). This suggests that PTN molecules were more likely to interact with the crowded coronas of PND<sub>34</sub>-b-PS<sub>14</sub>-C<sub>12</sub> micelles, than with individual PND<sub>34</sub>-C<sub>2</sub> chains in solution. In addition, only cross-peaks between the aromatic group of PTN and the isopropyl group of the NIPAm repeat unit of the self-assembled PND<sub>34</sub>-C<sub>12</sub> micelles were observed (Figure 4.13b), implying that interactions between PTN and diblock polymer PND<sub>34</sub>-b-PS<sub>14</sub>-C<sub>12</sub> micelles were stronger than the ones between PTN and self-assembled PND<sub>34</sub>-C<sub>12</sub> micelles.
Figure 4.11. (a) 2D $^1$H NOESY spectra of (a) 50 μg/mL PTN and 72 μg/mL PND$_{34}$-b-PS$_{14}$-C$_{12}$ and (b) 100 μg/mL NID and 144 μg/mL PND$_{34}$-b-PS$_{14}$-C$_{12}$ in deuterated PBS solution. The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution. Circled cross peaks indicate intermolecular polymer-drug interactions in solution for both model drugs. Chemical structures of PTN, NID, and diblock polymer with labeled protons are inserted.

Figure 4.12. 1D spectrum sliced at 7.48 ppm from the 2D $^1$H NOESY spectrum between 50 μg/mL phenytoin and 72 μg/mL PND$_{34}$-b-PS$_{14}$-C$_{12}$ in deuterated PBS solution.
Similarly, cross-peaks between the aromatic and methyl groups of NID and the isopropyl and methyl groups of PND$_{34}$-$b$-PS$_{14}$-$C_{12}$ were detected (Figure 4.11b). Both the isopropyl and methyl peaks of PND$_{34}$-$b$-PS$_{14}$-$C_{12}$ were observed in the 1D spectrum sliced at the NID methyl peak (i.e., 1.57 ppm) from the 2D $^1$H NOESY spectrum (Figure 4.14), indicating that NID molecules were in close spatial proximity with both NIPAm and DMA repeat units in the PND$_{34}$-$b$-PS$_{14}$-$C_{12}$ micelle corona. In contrast, only cross-peaks between the methyl groups of NID and the isopropyl groups of the self-assembled PND$_{34}$-$C_{12}$ micelles were detected, while no cross-peaks were observed between NID and individual PND$_{34}$-$C_{2}$ chains (Figure 4.15). Based on these results, both model drugs interacted most strongly with the diblock polymer PND$_{34}$-$b$-PS$_{14}$-$C_{12}$ micelles, followed by the self-assembled PND$_{34}$-$C_{12}$ micelles, and to the least extent with the individual PND$_{34}$-$C_{2}$ chains.
**Figure 4.14.** 1D spectrum sliced at 1.57 ppm from the 2D $^1$H NOESY spectrum between 100 μg/mL nilutamide and 144 μg/mL PND$_{34}$-b-PS$_{14}$-C$_{12}$ in deuterated PBS solution.

**Figure 4.15.** 2D $^1$H NOESY spectra between 100 μg/mL of nilutamide and 100 μg/mL of (a) PND$_{34}$-C$_2$ and (b) PND$_{34}$-C$_{12}$ in deuterated PBS solution.
The diffusion coefficients of both model drugs and a small molecule tracer (i.e., TSP) in the presence of the polymers at various concentrations were measured to further assess the drug-polymer interactions. PTN and NID concentrations were held at 50 and 100 µg/mL, respectively (with equal concentrations of TSP). The corresponding reduced diffusion coefficients \( (D/D_0, D/D_0 = 1 \text{ in the absence of polymer}) \) are plotted as a function of PND concentration in Figure 4.16. The diffusion coefficients of TSP were independent of the polymer type and concentration with both model drugs in all DOSY experiments (Figure 4.17), indicating that the polymer concentrations in this study were low enough such that any changes in diffusion of the small molecules were caused by specific interactions between the small molecules and polymers, rather than by changes in the macroscopic solution viscosity.\(^{113}\)

**Figure 4.16.** Reduced diffusion coefficients \( (D/D_0) \) of (a) PTN and (b) NID measured by DOSY in deuterated PBS solution as a function of the normalized PND concentration. PTN and NID concentrations are held at 50 and 100 µg/mL, respectively. The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution. Solid lines represent a two-state model that accounts for the diffusion of free and bound drug molecules, from which the indicated binding constants \( K_b \) were determined.
Figure 4.17. Reduced diffusion coefficients ($D/D_0$) of trimethylsilyl propionic acid sodium salt (TSP) in deuterated PBS solution with dissolved (a) phenytoin (PTN) and (b) nilutamide (NID) as a function of the normalized PND concentration. TSP concentrations are held at (a) 50 and (b) 100 µg/mL, respectively. The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution.

Both model drugs by themselves diffused at least one order of magnitude faster than the polymers (Figure 4.18), and the measured time-weighted diffusion coefficient ($D$) decreased when a fraction of the drug molecules was “bound” to the polymers due to favorable drug-polymer interactions. The molecular motions of both PTN and NID can be described by a single exponential decay (i.e., a single time-weighted $D$ value was obtained by fitting to Equation 2.5, Figure 4.18), indicating that the exchange time between the free and “bound” drug molecules was much shorter than the diffusion time of the DOSY experiments (i.e., $\Delta = 100$ ms in Equation 1).\textsuperscript{189,190} A two-state model, described in detail previously, was used to account for the partitioning of the drug into two species, as free and “bound” molecules in solution.\textsuperscript{113} By assuming the “bound” drug molecule has the same diffusion coefficient as the polymer ($D_{\text{polymer}}$), the fractions of the free drug ($p_{\text{free}}$) and “bound” drug ($p_{\text{bound}}$) can be estimated by the following equation (i.e., $p_{\text{free}} + p_{\text{bound}} = 1$):
\[ D = p_{\text{free}} D_{\text{drug,0}} + p_{\text{bound}} D_{\text{polymer}} \]  

(4.9)

where \( D_{\text{drug,0}} \) is the diffusion coefficient of free drug molecules. A binding constant \( (K_b) \), defined in Equation 4.10, was used to quantify and compare the drug-polymer interactions across all polymers:

\[ K_b = \frac{[\text{drug}]_{\text{bound}}}{[\text{drug}]_{\text{free}} [\text{repeat unit}]} \]  

(4.10)

where \([\text{drug}]_{\text{bound}}\) is the molar concentration of drug molecules bound to the polymer, \([\text{drug}]_{\text{free}}\) is the molar concentration of free drug molecules, and \([\text{repeat unit}]\) is the molar concentration of the PND repeat unit (by assuming the frozen polystyrene core is an inert component in the case of diblock polymer micelles).

**Figure 4.18.** Representative fits for the diffusion coefficient of (a) PTN (50 μg/mL), TSP (50 μg/mL), and PND_{34}-C_{12} (50 μg/mL) in deuterated PBS solution and (b) NID (100 μg/mL), TSP (100 μg/mL), and PND_{34}-C_{12} (100 μg/mL) in deuterated PBS solution.
The $K_b$ values between both model drugs and the investigated polymers were averaged over all concentrations and listed in Figure 4.16. The diffusion coefficients of both PTN and NID were nearly unaffected by the addition of PND$_{34}$-C$_2$, as indicated by the low $K_b$ values ($K_b = 3 \pm 14$ and $8 \pm 4$ for PTN and NID, respectively). This suggests that both model drugs have rather weak interactions with individual PND$_{34}$-C$_2$ chains, which is consistent with the absence of drug-polymer cross-peaks in the corresponding NOESY spectra (Figure 4.13 and Figure 4.15). For the diblock polymer micelles with the same corona block, the $K_b$ values of both PTN and NID increased systematically with the increasing polystyrene core block length. In fact, the $K_b$ value between PTN and PND$_{34}$-$b$-PS$_{14}$-C$_{12}$ ($K_b = 288 \pm 24$) was at least three times higher than the values of any NIPAm-based polymers reported in previous studies.$^{11,100}$ The increase of $K_b$ values correlates with the increase of the corona density values for these diblock polymer micelles ($\rho_{\text{corona}}$ in Table 4.2). Within experimental error, the $K_b$ values of PND$_{34}$-C$_{12}$ were equal between PTN ($K_b = 25 \pm 11$) and NID ($K_b = 29 \pm 7$). These values were surprisingly lower than those of the diblock polymer micelles, although the self-assembled PND$_{34}$-C$_{12}$ micelle has a comparable corona density to the PND$_{34}$-$b$-PS$_{14}$-C$_{12}$ micelle (Table 4.2). We speculate that the polystyrene at the core-corona interface strengthens the drug-polymer binding through favorable hydrophobic interactions. This is coincidently in agreement with the fact that the binding of these diblock polymer micelles was stronger with the more hydrophobic model drug PTN (i.e., PTN has lower aqueous solubility than NID in crystalline form). The impact of these drug-polymer interactions on the in vitro dissolution performance of both model drugs is discussed in the following section.

**In vitro Dissolution Assay Results**

To evaluate the efficacy of the polymers as excipients, polymers were spray dried with either PTN or NID and subjected to in vitro dissolution tests under non-sink conditions in PBS solution with 0.5 wt% of fasted simulated intestinal fluid powder
(FaSSIF) at 37 °C for 6 h, simulating conditions in the intestinal lumen. All dissolution experiments were prepared with a total drug concentration of 1000 µg/mL. The influence of polymer solution state assembly prior to spray drying on the dissolution and supersaturation maintenance of SDDs was investigated at 10 wt% loading of PTN. Selective and nonselective solvents for the PND corona block were used to form micelles and free chains prior to the spray drying process, respectively. The target PTN concentration was approximately 21 times higher than its equilibrium solubility in its crystalline form in PBS/FaSSIF solution at 37 °C (indicated as a dashed line in Figure 4.20). The enhancement factor (EF), defined in Equation 6 as the ratio of the area under the dissolution concentration–time curve (AUC) of the SDD to the AUC of the crystalline drug over a 6 h experimental period, was used to quantify dissolution performance for comparison purposes:

\[
EF = \frac{\text{AUC}(360 \text{ min})_\text{SDD}}{\text{AUC}(360 \text{ min})_\text{crystalline drug}}
\]  

THF was chosen as a non-selective solvent for the diblock polymers when spray drying with PTN. Upon direct dissolution in THF, all polymers showed a narrowly distributed single peak with a hydrodynamic radius of approximately 4 nm, indicating free chain conformations (Figure 4.19 and Table 4.4). The dissolution concentration–time curves for SDDs of the polymers spray dried from THF, along with a leading commercial excipient control reported in our previous work (i.e., HPMCAS), are shown in Figure 4.20a. Ricarte et al. have recently shown that maintenance of high PTN supersaturation directly correlated with the existence of amorphous nanoparticles containing PTN and HPMCAS, as visualized by cryo-TEM and quantified by SAXS. However, the PTN supersaturation level dropped quickly after the initial rapid dissolution as HPMCAS
failed to inhibit drug nucleation and crystal growth. In this study, PND<sub>34</sub>-C<sub>12</sub> and PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub> showed superior dissolution performance than other polymers, as both polymers achieved a rapid PTN release and maintained the supersaturation near the highest achievable concentration throughout the 6 h experimental period (C<sub>360min</sub> = 965 ± 27 and 979 ± 2 µg/mL for PND<sub>34</sub>-C<sub>12</sub> and PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub>, respectively). This is consistent with our previous results that polymeric excipients with self-micellizing capability often had higher enhancement factors, since both PND<sub>34</sub>-C<sub>12</sub> and PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub> can self-assemble into micelles after being directly dissolved in PBS solution (Figure 4.2 and Figure 4.21). Upon direct dissolution in PBS solution, PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub> self-assembled into micelles. As shown in Figure 4.21, the two peaks in DLS were interpreted as large aggregates and micelles. The peaks corresponding to large aggregates (R<sub>h1</sub> = 115 nm) and micelles (R<sub>h2</sub> = 23 nm) were obtained by fitting to a biexponential decay model, defined as:

\[
\langle g^{(2)}(\tau) \rangle = B + (A_1 e^{-2\Gamma_1 \tau} + A_2 e^{-2\Gamma_2 \tau})^2
\]

(4.11)

where \(\Gamma_1\) and \(\Gamma_2\) are the decay rates of the two modes, \(\tau\) is the delay time, \(A_1\) and \(A_2\) are the scattering intensity-weighted prefactors for the two decays and \(B\) is the baseline. Autocorrelation functions along with the best fits to Equation 4.11 and the corresponding decay rates \(\Gamma\) versus \(q^2\) plot are shown in Figure 4.22. The self-assembled PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub> micelles (i.e., R<sub>h2</sub>) had comparable hydrodynamic radii to those prepared by the cosolvent method (Figure 4.2 and Table 4.1). We speculate that the large aggregates (i.e., R<sub>h1</sub>) made a negligible contribution to the dissolution performance, due to their low number density compared to the micelles. At 10 wt% PTN loading, PND<sub>34</sub>-C<sub>2</sub> free chains achieved a dissolution profile comparable to PND<sub>34</sub>-C<sub>12</sub> and PND<sub>34</sub>-b-PS<sub>2</sub>-C<sub>12</sub> micelles.
within experimental error. In contrast, PND$_{34}$-$b$-PS$_9$-$C_{12}$ and PND$_{34}$-$b$-PS$_{14}$-$C_{12}$ did not dissolve well in PBS solution due to the longer hydrophobic polystyrene blocks, and therefore, they failed to achieve a high PTN supersaturation. The supersaturation level was lower for the diblock polymer with the longer hydrophobic polystyrene block (i.e., PND$_{34}$-$b$-PS$_{14}$-$C_{12}$), possibly because of its lower solubility in PBS solution as free chains.

![Figure 4.19](image)

**Figure 4.19.** Hydrodynamic radius distributions of the polymer samples at 20 mg/mL in THF, measured at the 90° scattering angle at 27 °C. All samples were prepared by direct dissolution in THF.

<table>
<thead>
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<th>$R_h$ (nm)</th>
<th>dispersity ($\mu_2/\Gamma^2$)</th>
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<tr>
<td>PND$_{34}$-C$_2$</td>
<td>4.2</td>
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</tr>
<tr>
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</tr>
<tr>
<td>PND$<em>{34}$-$b$-PS$</em>{14}$-$C_{12}$</td>
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</tr>
<tr>
<td>PND$_{34}$-$b$-PS$<em>9$-$C</em>{12}$</td>
<td>4.3</td>
<td>0.1</td>
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<tr>
<td>PND$_{34}$-$b$-PS$<em>2$-$C</em>{12}$</td>
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<td>0.09</td>
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Note: all samples prepared by direct dissolution
Results obtained by fitting to a second-order cumulant expansion model
Figure 4.20. PTN dissolution concentration–time curves for SDDs with drug loadings of 10 wt% at 37 °C when polymers were (a) directly dissolved in THF or (b) dissolved in MeOH before spray drying (the diblock polymers were prepared by the cosolvent method and lyophilized before dissolution in MeOH). Dashed lines indicate the measured equilibrium solubility of the crystalline PTN in PBS/FaSSIF solution.

Figure 4.21. Hydrodynamic radius distributions of PND₃₄-b-PS₂-C₁₂ upon direct dissolution in PBS solution at 10 mg/mL, measured at the 90° scattering angle at 37 °C.
Figure 4.22. (a) Autocorrelation function and (b) decay rate $\Gamma$ versus $q^2$ for DLS data of 10 mg/mL PND$_{34}$-$b$-PS$_2$-C$_2$ in PBS solution at 37 °C upon direct dissolution by using a biexponential decay model fit.

To maintain the micelle morphology of the diblock polymers with frozen polystyrene cores before spray drying, MeOH was chosen as a selective solvent for the PND corona block. Since PND$_{34}$-C$_2$ and PND$_{34}$-C$_{12}$ were both free chains in either MeOH (Figure 4.23) or THF (Figure 4.19), their corresponding dissolution profiles were the same within experimental error (Figure 4.20), regardless of the spray drying solvent. With a rather short polystyrene block, PND$_{34}$-$b$-PS$_2$-C$_{12}$ showed two populations in MeOH, mostly free chains with a small fraction of larger aggregates (Figure 4.23). Upon dissolution in the PBS solution, PND$_{34}$-$b$-PS$_2$-C$_{12}$ also achieved nearly a full release of PTN by spray drying with MeOH (Figure 4.20b). This was attributed to its self-micellizing capability in PBS solution (Figure 4.21). Unlike in THF, lyophilized PND$_{34}$-$b$-PS$_9$-C$_{12}$ and PND$_{34}$-$b$-PS$_{14}$-C$_{12}$ micelles prepared by the cosolvent method maintained their well-defined micelle morphology upon dissolution in MeOH (Figure 4.23). The $R_h$ and dispersity ($\mu^2/\Gamma^2$) of polymers in MeOH were obtained by fitting to a second-order cumulant expansion model (Equation 3.1) and are summarized in Table 4.5 (except for PND$_{34}$-$b$-PS$_2$-C$_{12}$). PND$_{34}$-$b$-PS$_2$-C$_{12}$ showed two populations in MeOH (Figure 4.23). The peaks
corresponding to large aggregates \( (R_{h1}) \) and free chains \( (R_{h2}) \) were fit to a biexponential decay model (Equation 4.11) and the results are shown in Table 4.5. Autocorrelation functions along with the best fits to Equation 4.11 and the corresponding decay rates \( \Gamma \) versus \( q^2 \) plot are shown in Figure 4.24. After spray drying, PTN molecules were presumably loaded in the micelle corona, rather than the frozen polystyrene core, for PND\textsubscript{34}-b-PS\textsubscript{9}-C\textsubscript{12} and PND\textsubscript{34}-b-PS\textsubscript{14}-C\textsubscript{12}.

**Figure 4.23.** Hydrodynamic radius distributions of the polymer samples at 20 mg/mL in MeOH, measured at the 90° scattering angle at 27 °C. The diblock polymers were prepared by titration, dialysis, and lyophilization before dissolution in MeOH.

**Table 4.5.** Hydrodynamic radius and dispersity of the polymers in MeOH

<table>
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<th>( R_h ) (nm)</th>
<th>dispersity (( \mu^2/\Gamma^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{a}\text{PND}_{34}\text{-C}_2)</td>
<td>3.9</td>
<td>0.04</td>
</tr>
<tr>
<td>(^{a}\text{PND}<em>{34}\text{-C}</em>{12})</td>
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<td>0.07</td>
</tr>
<tr>
<td>(^{b}\text{PND}<em>{34}\text{-b-PS}</em>{14}\text{-C}_{12})</td>
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</tr>
<tr>
<td>(^{b}\text{PND}<em>{34}\text{-b-PS}</em>{9}\text{-C}_{12})</td>
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<td>0.1</td>
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<table>
<thead>
<tr>
<th></th>
<th>( R_{h1} ) (nm)</th>
<th>( R_{h2} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{b}\text{PND}<em>{34}\text{-b-PS}</em>{2}\text{-C}_{12})</td>
<td>82</td>
<td>4.3</td>
</tr>
</tbody>
</table>

\(^{a}\) prepared by direct dissolution

\(^{b}\) prepared by the cosolvent method before dissolution
Figure 4.24. (a) Autocorrelation function and (b) decay rate $I'$ versus $q^2$ for DLS data of 20 mg/mL PND$_{34}$-b-PS$_2$-C$_2$ in MeOH at 27 °C (prepared by the cosolvent method before dissolution), by using a biexponential decay model fit.

As a result, the dissolution performance of the two diblock polymers was improved significantly by pre-forming the micelles before spray drying (Figure 4.20b), compared to spray drying as free chains in THF (Figure 4.20a). For the diblock polymer with the longest polystyrene chain (PND$_{34}$-b-PS$_{14}$-C$_{12}$), full release of PTN was achieved initially ($C_{40\text{min}} = 1004 \pm 27 \mu g/mL$), however, the PTN supersaturation level dropped slowly afterwards due to crystallization. This might be due to the induced nucleation and growth of the sequestered PTN molecules as a result of their high local concentration in the micelle corona. In fact, a higher drug-polymer binding constant from the DOSY experiments (Figure 4.16) indicated a higher fraction of PTN was stored in the micelle corona. Induced nucleation and growth of the sequestered PTN would be most likely to occur in the micelles with the highest binding constant (i.e., PND$_{34}$-b-PS$_{14}$-C$_{12}$). Nevertheless, by forming the micelles prior to spray drying in MeOH, PND$_{34}$-b-PS$_9$-C$_{12}$ (EF = 19.2 ± 0.6) and PND$_{34}$-b-PS$_{14}$-C$_{12}$ (EF = 16.2 ± 0.3) still significantly outperformed the commercial excipient HPMCAS (EF = 5.0 ± 0.3) in supersaturating PTN. At a higher loading of 25 wt%, the initial supersaturation level was lower for all the
polymers (Figure 4.25a), presumably due to the lower polymer-to-drug ratio. With 25 wt% PTN, the micelle-forming PND$_{34}$-C$_{12}$ delivered a considerably higher supersaturation than free chains of PND$_{34}$-C$_{2}$ (Figure 4.25a). The difference between PND$_{34}$-C$_{12}$ and PND$_{34}$-C$_{2}$ was also observed at a longer time point after dissolution (i.e., C$_{24\text{hr}}$ = 879 ± 32 and 287 ± 1 µg/mL for PND$_{34}$-C$_{12}$ and PND$_{34}$-C$_{2}$, respectively) even with only 10 wt% of PTN (Figure 4.25b). Both observations suggest that the PND$_{34}$-C$_{12}$ micelle is a better crystallization inhibitor than PND$_{34}$-C$_{2}$ free chains at equal polymer molecular weight and concentration.

![Figure 4.25.](image_url)  

**Figure 4.25.** Phenytoin dissolution concentration–time curves for SDDs at 37 °C with drug loadings of (a) 25 wt% and (b) 10 wt% (with 24 h data). Polymers were dissolved in MeOH before spray drying (the diblock polymers were prepared by the cosolvent method before dissolution in MeOH). Dashed lines indicate the equilibrium solubility of the crystalline phenytoin in PBS/FaSSIF solution.

The polymer efficacy as an excipient in SDDs was also investigated by using NID as a model drug at both 10 and 25 wt% loading (Figure 4.26). All the diblock polymer micelles were formed by the cosolvent method and lyophilized before dissolution in MeOH for spray drying. The total NID concentration (i.e., 1000 µg/mL) was approximately 7.9 times higher than its measured equilibrium solubility in PBS/FaSSIF solution at 37 °C, indicated by the dashed line in Figure 4.26. With 25 wt% NID, PND$_{34}$-
C_{12} achieved its highest supersaturation level at 40 min \( (C_{40\text{min}} = 845 \pm 32 \ \mu g/mL) \), which then started to decrease after 90 min due to drug crystallization. At equal NID loading of 25 wt%, the PND_{34}-C_{2} free chains had lower supersaturation at all time points, compared to the self-assembled PND_{34}-C_{12} micelles. PND_{34}-C_{2} and PND_{34}-C_{12} had either comparable or slightly better dissolution performance \( (EF = 5.1 \pm 0.6 \ \text{for PND}_{34}-C_{2} \ \text{and} \ 6.0 \pm 0.2 \ \text{for PND}_{34}-C_{12}) \) compared to HPCMAS \( (EF = 5.3 \pm 0.1) \), respectively. All three diblock polymer micelles had inferior dissolution performance compared to HPMCAS with 25 wt% NID. For these diblock polymer micelles, the supersaturation level of NID decreased due to drug nucleation and crystal growth after reaching an initially high level, and the drug desupersaturation was more rapid for the micelles with a larger polystyrene core. A similar trend was also observed with 10 wt% NID loading (Figure 4.26b), although each polymer had a slightly better supersaturation maintenance due to the higher polymer to drug ratio. At 10 wt% loading, both PND_{34}-C_{2} and PND_{34}-C_{12}, along with HPCMAS, were effective in maintaining the supersaturation of NID over the entire 6 h experimental period.

**Figure 4.26.** NID dissolution concentration–time curves for SDDs with drug loadings of (a) 25 wt% and (b) 10 wt% at 37 °C. All polymers were dissolved in MeOH before spray drying. The diblock polymer solutions were prepared by the cosolvent method and lyophilized before dissolution in MeOH. Dashed lines indicate the measured equilibrium solubility of the crystalline NID in PBS/FaSSIF solution.
4.4 Discussion

The predicted amorphous solubility of phenytoin was estimated to be about 2000 μg/mL at 37 °C in our previous work. The ΔH_f and T_m values for nilutamide were reported by Trasi and Taylor to be 98 J/g and 153 °C, respectively. Based on these values and a measured equilibrium concentration of crystalline nilutamide of 127 μg/mL in this work, the predicted amorphous solubility of nilutamide is approximately 1400 μg/mL at 37 °C, according to Equations 4.12 and 4.13 below (assuming γ ≈ 1),

\[ c = γc_{eq}\exp\left(\frac{ΔG}{RT}\right) \]

\[ ΔG = \frac{ΔH_f(T_m - T)}{T_m^2} \]

where \( c \) is the amorphous drug solubility, \( γ \) is the activity coefficient of amorphous drug saturated with water, \( c_{eq} \) is the equilibrium concentration of the crystalline drug, \( ΔG \) is the free energy of crystallization, \( R \) is the universal gas constant, \( T \) is the actual temperature, \( ΔH_f \) is the enthalpy of fusion, and \( T_m \) is the drug melting temperature. The octanol-water partition coefficient (log P) and free energy of crystallization at 37 °C (ΔG) for phenytoin and nilutamide are summarized in Table 4.6.

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<th>Drug</th>
<th>log P</th>
<th>ΔG (kJ/mol)</th>
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<tbody>
<tr>
<td>phenytoin</td>
<td>2.47</td>
<td>9.9</td>
</tr>
<tr>
<td>nilutamide</td>
<td>2.14</td>
<td>6.2</td>
</tr>
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</table>

Table 4.6. Summary of octanol-water partition coefficient (log P) and free energy of crystallization at 37 °C (ΔG)
To compare the dissolution performance of the excipients for the two different model drugs, the enhancement factors for the SDDs with either PTN or NID at both drug loadings are summarized in Figure 4.27. PTN is not only a more hydrophobic compound than NID (i.e., higher octanol-water partition coefficient, log $P$), but it is also a stronger crystallizer, as evidenced by its higher free energy of crystallization (Table 4.6). Therefore, the maximum possible enhancement factor of PTN was higher than that of NID at equal target drug concentration (i.e., 1000 µg/mL). Surprisingly, the diblock polymers achieved higher enhancement factors relative to the maximum possible value with PTN than NID at 10 wt% drug loading, despite the fact that PTN has a much higher thermodynamic driving force to crystallize than NID at equal supersaturated concentration, as predicted by the Hoffman equation (Equation 4.13).\textsuperscript{166} This result is attributed to the micelle corona sequestration mechanism proposed previously, whereby hydrophobic drugs can be solubilized in the crowded corona region of the micelle, in the form of drug-rich amorphous nanoparticles.\textsuperscript{11,54} PTN may have a stronger affinity for the slightly hydrophobic micelle corona region compared to NID, due to its higher hydrophobicity. Such affinity is consistent with the cross-peaks observed in the NOESY spectra (Figure 4.11), most likely due to hydrophobic interactions between the drug and the micelle corona. Concurrently, the diblock polymer micelles had higher binding constants with PTN than with NID based on the DOSY results (Figure 4.16), resulting in a better dissolution performance (i.e., higher EF in Figure 4.27) with PTN than with NID. In contrast, HPMCAS (as the control) had the lowest enhancement factor with PTN among all the polymers, while outperforming most of the polymers with NID (Figure 4.27). The polymer micelles may be more effective in stabilizing the more hydrophobic and rapidly crystallizing drugs (e.g., PTN) through the corona sequestration mechanism, and therefore, the micelle corona loading strategy can be used as a replacement and/or complement to HPMCAS to improve the dissolution performance of these drugs. In
addition, the enhancement factors were much lower at a higher drug loading of 25 wt% for all the polymers, presumably due to the inefficient inhibition of drug nucleation and crystal growth at a lower polymer to drug ratio.

Figure 4.27. Enhancement factor (EF) as calculated at 360 min from the dissolution data of SDDs with 10 and 25 wt% loadings of (a) PTN and (b) NID. All polymers were dissolved in MeOH before spray drying (the diblock polymers were prepared by the cosolvent method and lyophilized before dissolution in MeOH). Maximum possible enhancement factors, indicated by the dash lines, are 21 and 7.9 for PTN and NID, respectively.

The predicted amorphous solubilities of both PTN (~ 2000 μg/mL) and NID (~ 1400 μg/mL) are above the target drug concentrations (i.e., 1000 μg/mL) during the dissolution
experiments. Taylor and Zhang have comprehensively discussed the “reservoir” effect of liquid-liquid phase separation in a recent review, whereby a supersaturated aqueous solution can be achieved in equilibrium with drug-rich nanodroplets. These drug-rich nanodroplets can maintain the drug supersaturation level by replenishing the absorbed drug. Recent work from our group has also shown that high drug supersaturation is directly correlated with the formation of presumably drug-rich nanoparticles. Although liquid-liquid phase separation is unlikely to occur when the supersaturated concentrations of PTN and NID are below their amorphous solubility, a similar “reservoir” concept was demonstrated in this study by using the crowded corona region of the micelle to stabilize the high drug supersaturation level through favorable drug-polymer intermolecular interactions. A good correlation between the strength of the drug-polymer interactions (i.e., $K_b$ in Figure 4.16) and the corona crowdedness ($\rho_{\text{corona}}$ in Table 4.2) was observed for the three diblock polymer micelles, suggesting that a more crowded micelle corona environment increases the drug affinity to the polymer. When the corona density of the diblock polymer micelles increased, a higher fraction of the free drug molecules in aqueous solution was “bound” to the polymers (e.g., the highest values of $p_{\text{bound}}$ were 0.42 and 0.38 for PTN and NID, respectively, with PND$_{34}$--b--PS$_{14}$--C$_{12}$), reflected by a higher $K_b$ value (Figure 4.16). However, this study demonstrated that a higher drug-polymer binding strength does not necessarily translate into a better dissolution performance, although it could be beneficial to inhibition of drug nucleation and crystal growth. We speculate that drug nucleation and crystal growth could occur not only in the aqueous solution but also in the micelle corona (e.g., induced crystallization due to its high concentration), and therefore, it is important to optimize the partitioning of free and “bound” drugs by tuning the corona density of the micelle. Thus, the maintenance of a high drug supersaturation level can be achieved when a delicate
balance between the free drug in the supersaturated aqueous phase and the “bound” drug in the micelle corona phase is reached.

Forming the micelle structure in a selective spray-drying solvent (i.e., MeOH in this study) was important in order to load the drug molecules into the corona of the diblock polymer micelles, rather than into the core. The in vitro dissolution performance of the diblock polymers with longer hydrophobic polystyrene blocks (i.e., PND_{34}-b-PS_{9}-C_{12} and PND_{34}-b-PS_{14}-C_{12}) was significantly improved by spray drying with the pre-formed micelles in MeOH, rather than as free chains in THF (e.g., compare Figure 4.20a and b). Spray drying is a kinetically limited process, whereby the drug molecules are presumed to be kinetically trapped in the polymer micelle corona due to the rapid evaporation of solvent. The drug molecules are more likely to interact with the micelle corona when the solute concentration in MeOH is increased, which may be beneficial for drug encapsulation. However, there are practical limitations to the solute concentration achievable by the spray drying process. First, both the drug and polymer need to be molecularly dissolved prior to spray drying. Second, while a higher solute concentration provides high initial solute saturation, it will increase the particle size of the solid dispersion, resulting in slower solid dispersion dissolution.

The role of the polymer LCST is critical when designing polymer micelles as excipients for oral drug delivery applications. In this study, the cloud point of the micelle corona block (i.e., 40-41 °C) was tuned to be slightly above body temperature (i.e., 37 °C) by incorporating sufficient hydrophilic monomer (i.e., 33 mol% of DMA), based on the high throughput screening work performed by Ting and coworkers. The drug-corona interaction is speculated to be stronger (i.e., higher $K_b$ value) with increasing mole fraction of the more hydrophobic monomer (i.e., NIPAm). Therefore, there exists a delicate balance between hydrophobic and hydrophilic monomers wherein the micelles can provide a slightly hydrophobic corona region to sequester the hydrophobic drug.
molecules while still remaining soluble in aqueous solution at 37 °C. In addition, high $T_g$ and non-polar polystyrene core was chosen such that the dry polystyrene core was kinetically frozen in aqueous solution in order to focus on the drug-polymer interaction mechanism by avoiding micelle chain exchange. Results presented in this study and previous work indicate that the micelle corona sequestration mechanism can be broadly applied to a variety of hydrophobic drugs (i.e., PTN, NID, and probucol) to improve their dissolution and supersaturation maintenance. In contrast, the release of trapped drug molecules in a typical core-loading method often requires an external stimulus-responsive mechanism (e.g., change of pH and/or temperature), otherwise, the bioavailability of the trapped drug would be compromised due to its low permeability through the gastrointestinal membrane. Good in vitro-in vivo correlation and no liver toxicity were observed in an animal study of P(NIPAm-co-DMA) polymers. Additionally, it has been shown that an FDA-approved styrene-containing triblock polymer (i.e., polystyrene-$b$-polyisobutylene-$b$-polystyrene) and polystyrene nanoparticles demonstrated good biocompatibility. Therefore, there is good reason to expect that biocompatibility will not be a concern in oral delivery applications for the polymers used in this work. The corona loading strategy showcased in this study, in parallel with other methods that successfully utilize drug-rich nanostructures, offers a promising platform for the design of efficient oral drug delivery formulations.

### 4.5 Conclusions

We demonstrate a simple yet powerful strategy to sequester hydrophobic drug molecules in the crowded corona region of well-defined micelles. The micelle corona serves as a reservoir to maintain high drug supersaturation, and such a corona loading strategy proves to be effective for two different hydrophobic drugs, PTN and NID. By
forming the micelle structures with a selective solvent (i.e., MeOH) rather than dissolving the diblock polymer as free chains in a non-selective solvent (i.e., THF) prior to spray drying, in vitro dissolution performance of the SDDs was improved with PTN. For these PND-\textit{b}-PS-C_{12} diblock polymer micelles, a more crowded corona environment was achieved by incorporating an inert polystyrene block with higher molecular weight, which resulted in a stronger drug-polymer interaction (i.e., higher $K_b$ value) for both PTN and NID. Nevertheless, higher PTN and NID supersaturation and maintenance during in vitro dissolution tests were observed for diblock polymer micelles with shorter polystyrene blocks, possibly due to induced nucleation and growth of the highly-concentrated drug molecules that were sequestered in the micelle corona. Micelles with self-assembling capability in aqueous solution (i.e., PND_{34}-C_{12} and PND_{34}-\textit{b}-PS_{2}-C_{12}) showed superior in vitro dissolution performance (i.e., higher enhancement factor) with both drugs despite their relatively lower drug-polymer “binding” strength. Therefore, the optimization of the micelle corona density plays an important role in balancing the fraction of free and “bound” drug molecules, for better drug supersaturation maintenance. This study reveals the importance of micelle solution-state assembly on both the drug-polymer interactions and the subsequent drug dissolution and supersaturation maintenance for oral drug delivery applications.
5. Chemically Crosslinked Nanogels

5.1 Introduction

It has been shown in Chapters 3 and 4 that a locally crowded polymer environment such as the corona region of a micelle, in which the corona polymer is relatively hydrophobic, favors the partitioning of the drug into the corona, and therefore can be used as a reservoir to store hydrophobic drug molecules for enhanced solubility. Polymer nanogels, also known as microgels, have certain extent of polymer crowding depending on degrees of chemical crosslinking and swelling, and therefore, also potentially can be utilized as a reservoir for the same purpose. Poly(N-isopropylacrylamide) (PNIPAm), one of the most extensively studied thermoresponsive polymers, has a lower critical solution temperature (LCST) in aqueous solution at approximately 32 °C, which is close to physiological temperature. This makes it a promising candidate for controlled delivery. Chemically crosslinked nanogels containing PNIPAm and its copolymers have been investigated for a variety of drug delivery routes including oral, injection, and transdermal. Similar to its linear counterpart, a PNIPAm-based nanogel swells in aqueous solution at low temperature but shrinks in size upon heating through the LCST, ultimately collapsing into a dense state with little water at temperatures well above the LCST. At low concentrations, a nanogel solution behaves like a dilute colloidal system, and the spherical nanogel particles are well-dispersed in the aqueous solution at an effective volume fraction. The size of the dispersed nanogel particles and the local concentration of the crosslinked polymer chains in each particle can be tuned by varying the crosslink density and the amount of surfactant during emulsion polymerization.

A series of PNIPAm nanogels with varying crosslink density, and several poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PND) nanogels containing different
co-monomer compositions, were synthesized via emulsion polymerization. Phenytoin, a Biopharmaceutical Classification System (BCS) Class II compound, was selected as the model hydrophobic drug to evaluate the performance of nanogels for drug solubility and dissolution enhancement. The physical properties of phenytoin were previously reported by Stella, et al. Commonly used polymer excipients such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl methylcellulose acetate succinate (HPMCAS) and polyvinylpirrolidone (PVP) were not able to maintain maximum phenytoin supersaturation due to rapid crystallization, despite moderate solubility enhancement. In this study, a near full release of the phenytoin loaded nanoparticles with sustained supersaturation over 6 hours was achieved using PNIPAm nanogels that were loaded with phenytoin by spray drying from solution into a solid amorphous powder of roughly micron sized particles. During dissolution, nanoparticles were observed and quantified by a combination of cryo-TEM and SAXS experiments. Dispersion of the nanoparticles was strongly dependent on the nanogel crosslink density, resulting in different dissolution profiles. The effect of the LCST on the nanogel structure and drug-nanogel partitioning, and the subsequent in vitro dissolution performance, were also investigated for a series of PND nanogels with various compositions.

5.2 Experimental Section

Details of the instruments used in this chapter and most of the experimental procedures were outlined in Chapter 2. This section summarizes the sample preparation, experimental details, and instrument processing parameters that are specific to this chapter.
Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted: N-isopropylacrylamide (NIPAm, ≥ 99%), N,N-dimethylacrylamide (DMA, 99%), N,N’-methylenebis(acrylamide) (BIS, 99%), sodium dodecyl sulfate (SDS, ≥ 99%), potassium persulfate (KPS, ≥ 99%), methanol (HPLC grade, 99.9%), acetonitrile (HPLC grade, 99.9%), water (HPLC grade), phenytoin (PTN, ≥ 99%), dimethyl sulfoxide-d₆ (DMSO-d₆, 99.9 atom% D, Cambridge Isotope Laboratories, Inc.), deuterium oxide (D₂O, 99.96 atom% D, Cambridge Isotope Laboratories, Inc.), 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt (TSP, 98 atom% D). Phosphate buffered saline (PBS, pH = 6.5) was prepared by using Milli-Q water, 82 mM sodium chloride (≥ 99.9%, Fisher), 20 mM sodium phosphate dibasic (99.5%), and 47 mM potassium phosphate monobasic (≥ 99%, J.T. Baker). Fasted simulated intestinal fluid powder (FaSSIF) was purchased from Biorelevant.

Dynamic Light Scattering (DLS)

For DLS, the normalized autocorrelation functions ($g^{(2)}$) of the scattered light intensity at scattering angles from 60° to 120° in 15° increments were fitted to a third-order cumulant model, defined by:

$$g^{(2)}(\tau) = B + \beta e^{-2\Gamma \tau} \left(1+\frac{\mu_2}{2} - \frac{\mu_3}{6}\right)^2$$

where $\Gamma$ is the mean decay rate, $\tau$ is the delay time, $B$ is the baseline of $g^{(2)}$, $\beta$ is the amplitude of the decay, and $\mu_2$ and $\mu_3$ are the second and third moments of the decay rate distribution, respectively.
Cryogenic Transmission Electron Microscopy (cryo-TEM)

Samples of vitrified nanogel solution and SDD dissolution media were prepared with a FEI Vitrobot vitrification system. Glowing discharge was performed on each 300-mesh carbon-coated lacey Formvar TEM grid (Ted Pella), which was then placed into the Vitrobot chamber held at 26 °C and 100% relative humidity. During \textit{in vitro} dissolution of the PNIPAm nanogel SDDs in PBS (pH = 6.5) at 22 °C, a 2.5 μL solution was extracted from the dissolution medium after 30 s of vortexing at various time points (t = 10, 40, 90, 180, and 360 min) and deposited onto the prepared TEM grid. The grid was blotted for 7 s with an instrument defined offset force of –2 and relaxation time of 3 s, then plunged into a pool of liquid ethane. The vitrified sample was stored in liquid nitrogen until imaging. Cryo-TEM images were taken with a FEI Tecnai G2 Spirit BioTWIN with an accelerating voltage of 120 kV at approximately –177 °C. The images were recorded by an Eagle 2k (4 megapixel) CCD camera with slight underfocus. Image analysis was performed by using ImageJ software (National Institutes of Health, MD, USA).

Small-Angle X-ray Scattering (SAXS)

SAXS experiments were performed on the DND-CAT 5-ID-D beamline at the Advanced Photon Source, Argonne National Laboratory. SDDs were loaded into 3.7 mL glass vials and 3.6 ml of PBS solutions (pH = 6.5) were added to achieve a target phenytoin concentration of 1000 μg/mL (t = 0 min). These vials were vortexed for 1 min and incubated in an aluminum vial holder at 22 °C. At various time points (t = 10, 20, 40, 90, 180, and 360 min), an approximately 0.1 mL aliquot of dissolution media was collected after 30 s of vortexing and transferred into 1.5 mm quartz capillary tubes. The vials were returned to the aluminum holder and incubated at 22 °C until the next time point. The capillary tubes were placed in a multi-capillary holder. Isotropic 2D scattering
patterns were collected immediately after the closure of the hutch door on a Rayonix CCD area detector with an exposure time of 2 s, and azimuthally integrated into 1D data of intensity (arbitrary units) vs scattering vector $q$ ($\text{Å}^{-1}$). Background was removed by subtracting the PBS solvent reference scattering profile from the 1D data of the polymer nanogel and dissolution media solution. The resulting intensity data were analyzed using the SASView software (version 4.1.2).

The scattering intensity ($I$) in dilute solution is a function of the form factor $P(q)$ and background ($B$, i.e., scattering intensity from PBS solution and capillary tube), as shown in Equation 5.2. An arbitrary prefactor ($A$) was used because the SAXS data were not reduced to an absolute scale.

$$I = A \cdot \int_0^\infty P(q)D(R)dR + B \quad (5.2)$$

The SAXS patterns from the dissolution media for 10 wt% phenytoin SDDs with PNIPAm nanogels were fit to a spherical form factor with a Gaussian distribution $D(R)$:

$$P(q) = \left[ 3 \frac{\sin(qR) - qr \cos(qR)}{(qR)^3} \right]^2 \quad (5.3)$$

where $q$ is the scattering vector and $R$ is the radius of the sphere.

The SAXS patterns of the PNIPAm nanogels were fit to a fuzzy spherical form factor with a Gaussian distribution, as shown below:

$$P(q) = \left[ 3 \frac{\sin(qR) - qr \cos(qR)}{(qR)^3} \exp \left( -\frac{(q\sigma_{\text{surf}})^2}{2} \right) \right]^2 \quad (5.4)$$
where $\sigma_{\text{surf}}$ is used to describe “fuzziness” of the surface interface (details discussed by Stieger et al.)\textsuperscript{208,212} The Gaussian distribution $D(R)$ is defined as:

$$D(R) = \frac{1}{\sigma \sqrt{2\pi}} \exp\left(-\frac{(R - R_{\text{mean}})^2}{2\sigma^2}\right)$$

(5.5)

where $R_{\text{mean}}$ is the mean radius of the distribution and $\sigma$ is the standard deviation of the radius. The polydispersity ($PD$) of the radius is:

$$PD = \frac{\sigma}{R_{\text{mean}}}$$

(5.6)

5.3 Results

**PNIPAm Nanogel Characterization**

Three PNIPAm nanogels with varying crosslink density (0.5, 2.5, and 5.0 mol% BIS) were synthesized via emulsion polymerization (see Figure 2.3 and Figure 5.1 for details).\textsuperscript{129,130} Hydrodynamic radius and size distributions of the PNIPAm nanogels in PBS (pH = 6.5, $T = 22$ °C) were obtained using dynamic light scattering (DLS), as shown in Figure 5.2a. Table 5.1 summarizes the mean hydrodynamic radius ($R_h$) and dispersity ($\mu_2/\Gamma^2$), calculated by fitting the autocorrelation functions at five different scattering angles to a third-order cumulant model (Figure 5.3). All three nanogel solutions show a narrow single peak (i.e., low $\mu_2/\Gamma^2$). The mean $R_h$ of the PNIPAm nanogels decreased as the crosslink density increased, indicating that the nanogels were less swollen in PBS with a higher mol% of BIS. Small-angle X-ray scattering (SAXS) patterns and cryo-TEM images of the pure nanogels in PBS are shown in Figure 5.2b and c, respectively.
Figure 5.1. $^1$H NMR spectra of PNIPAm nanogels (after dialysis, lyophilization, drying and re-dissolution in D$_2$O) and pure SDS in D$_2$O.

The SAXS patterns were fit to a “fuzzy sphere” model with a Gaussian size distribution, which accounts for both the nanogel dispersity and a gradually decaying (smeared) radial density profile at the nanogel interface.$^{208,212}$ Fitted radii from SAXS (i.e., $R_{\text{SAXS}}$) and radii obtained from the cryo-TEM images (i.e., $R_{\text{TEM}}$) of the PNIPAm nanogels are summarized in Table 5.1. Within experimental uncertainty $R_{\text{SAXS}}$ is independent of the nanogel crosslink density. However, $R_{\text{TEM}}$ varies between 90±7 and 57±7 nm from the lowest to highest crosslink density; both sets of values are smaller than the corresponding $R_h$, as expected. We interpret the invariant SAXS radii as a reflection of a polymer concentration within the particles, hence crosslink density, which varies with radial distance from the center of the particles, i.e., the particles do not swell uniformly. This likely explains the minor but systematic deviation of the model fits to the SAXS data at higher values of scattering wavevector $q$ in Figure 5.2b. Differences in the overall polymer concentration are clearly manifested by variations in the contrast seen in
the cryo-TEM images (Figure 5.2c). The particles containing 5 mol% BIS are significantly darker than those with 2.5 and 0.5 mol% BIS. Moreover, a hint of a core-shell structure in the most highly crosslinked particles reinforces the deduction regarding inhomogeneous crosslinking. Overall, these characterization results confirm that populations of spherical particles with relatively uniform size distributions are formed by the emulsion polymerization method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_h^a$ (nm)</th>
<th>$\mu_2/T^2^a$</th>
<th>$R_{SAXS}^b$ (nm)</th>
<th>$R_{TEM}^c$ (nm)</th>
<th>$\rho_{nanogel}^d$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mol% BIS</td>
<td>133</td>
<td>0.05</td>
<td>59 ± 8</td>
<td>90 ± 8</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>2.5 mol% BIS</td>
<td>109</td>
<td>0.07</td>
<td>58 ± 7</td>
<td>67 ± 7</td>
<td>72 ± 1</td>
</tr>
<tr>
<td>5.0 mol% BIS</td>
<td>100</td>
<td>0.06</td>
<td>55 ± 7</td>
<td>57 ± 4</td>
<td>109 ± 5</td>
</tr>
</tbody>
</table>

Determined by $^a$DLS, $^b$SAXS, $^c$cryo-TEM, and $^d$viscometry.
Figure 5.2. (a) Hydrodynamic radius distributions of the PNIPAm nanogels at 0.5 mg/mL in PBS at 22 °C at 90° scattering angle. (b) SAXS patterns and (c) cryo-TEM images of PNIPAm nanogels at 9 mg/mL in PBS at 22 °C. The SAXS profiles are shifted vertically by factors of 10 for clarity. The solid lines in (b) are best fits to a fuzzy sphere model with a Gaussian distribution. Scale bars in (c) equal 100 nm.
The relative viscosity ($\eta_{\text{rel}}$) of PNIPAm nanogels in PBS is plotted as a function of concentration in Figure 5.4. For moderately dilute suspensions, $\eta_{\text{rel}}$ can be expressed as a function of the nanogel effective volume fraction ($\phi_{\text{eff}}$), as shown in Equation 5.7.\textsuperscript{129,213,214} The nanogel particle density ($\rho_{\text{nanogel}}$) was calculated by fitting the $\eta_{\text{rel}}$ data to Equations 5.7-8 and the results are summarized in Table 5.1. Consistent with the DLS and cryo-TEM results, $\rho_{\text{nanogel}}$ increased as the crosslink density increased (i.e., approximately a 3-fold increase from 0.5 to 5.0 mol% BIS). This indicates that on average a locally more concentrated NIPAm environment in the nanogel particle was achieved by incorporating a higher mol% of BIS during the emulsion polymerization.
Figure 5.4. Relative viscosity of the PNIPAm nanogels in PBS at 22 °C as a function of the polymer concentration. Solid lines indicate best fit of the data to Equations 5.7 and 5.8.

\[
\eta_{\text{rel}} = 1 + 2.5\varphi_{\text{eff}} + 5.9\varphi_{\text{eff}}^2
\]  

(5.7)

\[
c = \varphi_{\text{eff}} \rho_{\text{nanogel}}
\]  

(5.8)

Dissolution of Phenytoin and PNIPAm Nanogel SDDs

2D NOESY experiments were performed to investigate phenytoin-nanogel interactions in D$_2$O solution. Nanogels were dissolved in D$_2$O (1000 μg/mL) and phenytoin in DMSO (5 mg/mL) was added to yield a drug concentration of 50 μg/mL. Cross peaks between the phenyl groups (i.e., 7.4–7.5 ppm) of phenytoin and isopropyl groups (i.e., 1.1–1.2 ppm) of the PNIPAm nanogel (5.0 mol% BIS) were observed as shown in Figure 5.5, indicating that these two groups were in close spatial proximity. At equal phenytoin and nanogel concentrations, similar cross peaks were also observed for PNIPAm nanogels with different crosslink densities (Figure 5.6). No phenytoin-nanogel cross peaks were observed when reducing the PNIPAm nanogel (5.0 mol% BIS)
concentration from 1000 μg/mL to 50 μg/mL (Figure 5.7), which could be attributed to less partitioning of the phenytoin molecules to the nanogels, and/or the weaker signal strength at lower nanogel-to-phenytoin ratios.

**Figure 5.5.** 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 1000 μg/mL of PNIPAm nanogel (5.0 mol% BIS) in D$_2$O. Cross peak (circled) between the aromatic peaks around 7.4–7.5 ppm of phenytoin and the isopropyl peaks around 1.1–1.2 ppm of PNIPAm nanogel indicates intermolecular drug-polymer interactions in solution. Chemical structures of phenytoin and PNIPAm nanogel with labeled protons are inserted.
Figure 5.6. 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 1000 μg/mL of PNIPAm nanogel with (a) 0.5 and (b) 2.5 mol% BIS. Chemical structures of phenytoin and PNIPAm with labeled protons are shown in Figure 5.5. Cross peaks are circled.
Figure 5.7. 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 50 μg/mL of PNIPAm nanogel (5.0 mol% BIS) in D$_2$O. Chemical structures of phenytoin and PNIPAm with labeled protons are shown in Figure 5.5.

To further evaluate drug solubilization enhancement from a solid dispersion formulation, PNIPAm nanogel and phenytoin were spray dried from methanol (2 wt% total solid content) resulting in drug loadings of 10 and 25 wt%. Representative scanning electron microscopy (SEM) images of the spray dried dispersions (SDDs), shown in Figure 5.8, indicate that neither the bulk morphology nor the particle size of the SDDs depends on the nanogel crosslink density. At both drug loadings, featureless diffraction patterns were observed in synchrotron wide-angle X-ray scattering (WAXS) (Figure 5.9), demonstrating that phenytoin in the SDDs was amorphous.
Figure 5.8. SEM images taken for SDDs of PNIPAm nanogels with (a) 5.0, (b) 2.5, (c) 0.5 mol% of BIS at 10% phenytoin loading. Scales bars equal to 1 μm.

Figure 5.9. Wide angle X-ray scattering patterns of the SDDs of the PNIPAm nanogels at phenytoin loading of 10 wt% and 25 wt%; $q$ is the scattering vector.

Following the widely-used in vitro microcentrifuge drug dissolution procedure,$^{23,24}$ SDDs with 10 wt% drug loading were dissolved in PBS at 22 °C with a target phenytoin concentration of 1000 μg/mL (i.e., ~37 times higher than its solubility in crystalline form).
The dissolution media were imaged using cryo-TEM (Figure 5.10; more images are shown in Figure 5.11). (Here we note that SDDs of the pure nanogels (i.e., no drug) yielded the same size nanoparticles as are listed in Table 5.1 following dissolution in PBS as evidenced by DLS (Figure 5.12)). For the PNIPAm nanogel with 5.0 mol% BIS, well-dispersed spherical nanoparticles, presumably nanogels with encapsulated drugs, were observed in the SDD dissolution media, and their sizes are summarized in Table 5.2. The average size of the nanoparticles (radius ~44 nm) remained the same throughout the dissolution process, and was significantly smaller than the pure nanogel in PBS ($R_{TEM} = 59 \pm 6 \text{ nm}$ in Figure 5.10). For PNIPAm nanogels with 0.5 and 2.5 mol% BIS, a mixture of much larger particles ($R >> 500 \text{ nm}$) and spherical nanoparticles was observed at the early time points during dissolution. The larger particles (i.e., $R >> 500 \text{ nm}$) appear to be disintegrating SDDs that are composed of the smaller spherical nanoparticles. Table 5.2 lists the average sizes of the smaller spherical nanoparticles for all three sets of PNIPAm nanogels. Within experimental error the radius of the nanoparticles is independent of the dissolution time and the nanogel crosslink density, in contrast with the pure nanogels, which decrease in size with increasing crosslink density. With 2.5 mol% BIS, the larger particles slowly disintegrated and dispersed spherical nanoparticles over time. In contrast, larger particles are still evident in the cryo-TEM from the 0.5 mole% BIS PNIPAm solution after 360 min.
Figure 5.10. Representative cryo-TEM images taken during dissolution in PBS at 22 °C for SDDs of PNIPAm nanogels with varying crosslink density (0.5, 2.5, 5.0 mol% of BIS) at 10 wt% phenytoin loading. Nanogel-only samples were dissolved at 9 mg/mL in PBS at 22 °C. Scale bars equal 200 nm.

Table 5.2. Cryo-TEM analysis of 10 wt% phenytoin in PNIPAm nanogels

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_{10\text{ min}}$ (nm)</th>
<th>$R_{90\text{ min}}$ (nm)</th>
<th>$R_{360\text{ min}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 mol% BIS</td>
<td>46 ± 5</td>
<td>44 ± 5</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>2.5 mol% BIS</td>
<td>44 ± 5</td>
<td>43 ± 4</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>0.5 mol% BIS</td>
<td>42 ± 4</td>
<td>43 ± 5</td>
<td>41 ± 5</td>
</tr>
</tbody>
</table>
Figure 5.11. Representative cryo-TEM images taken during dissolution in PBS solution (pH = 6.5) at 22 °C for SDDs of PNIPAm nanogels with varying cross-link density (0.5, 2.5, 5.0 mol% of BIS) at 10% phenytoin loading. Scale bars equal 200 nm.
Figure 5.12. Hydrodynamic radius distributions of the spray dried PNIPAm nanogel particles that were re-dissolved (after 1 min of vortexing) in PBS solution at 0.5 mg/mL (pH = 6.5, T = 22 °C) at 90° scattering angle.

Table 5.3. Hydrodynamic radius and dispersity of the spray dried PNIPAm nanogels that were re-dissolved in PBS solution (pH = 6.5, T = 22 °C)

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_h$ (nm)</th>
<th>$\mu^2/I^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mol% BIS</td>
<td>138</td>
<td>0.1</td>
</tr>
<tr>
<td>2.5 mol% BIS</td>
<td>108</td>
<td>0.05</td>
</tr>
<tr>
<td>5.0 mol% BIS</td>
<td>101</td>
<td>0.07</td>
</tr>
</tbody>
</table>

We believe the difficulty in dispersing the 10 wt% 0.5 mol% BIS PNIPAm SDDs can be attributed to mutual interpenetration and entanglement of the dangling chains of the less crosslinked PNIPAm nanogels. Electron diffraction patterns were collected with an aperture selecting the agglomerated larger particles evident at 360 min. Featureless 2D patterns strongly suggest that these disintegrating larger particles contain amorphous phenytoin (Figure 5.13a-b). It has been previously shown that phenytoin rapidly crystallizes from PBS into long needle-like structures at such higher supersaturation in the absence of any polymer carriers,$^{11,168}$ resulting in poor oral bioavailability. We
conclude from these structural experiments that when encapsulated in the polymer nanogels, phenytoin remains amorphous after 6 h, in both the nanoparticles (i.e., $R \approx 43$ nm) and agglomerated macroparticles, as evidenced by the featureless 2D electron diffraction patterns (Figure 5.13c-d).

**Figure 5.13.** (a) Cryo-TEM image and (b) electron diffraction pattern taken during dissolution of 10 wt% SDD of PNIPAm nanogel with 0.5 mol% BIS in PBS solution at 22 °C at 360 min. (c) Cryo-TEM image and (d) electron diffraction pattern taken during dissolution of 10 wt% SDD of PNIPAm nanogel with 5.0 mol% BIS in PBS solution at 22 °C at 360 min. Red circles indicate position of selected area diffraction aperture during acquisition. Scale bars equal 200 nm in (a), (c) and 2 nm$^{-1}$ in (b), (d), respectively.
SAXS patterns of the dissolution media were collected using synchrotron X-rays (PBS background was subtracted), and are shown in Figure 5.14a and Figure 5.15. These scattering patterns were quantitatively fit to a spherical form factor with a Gaussian size distribution and the corresponding radii of the spherical nanoparticles are plotted in Figure 5.14b (error bars indicate radius standard deviation obtained from the Gaussian distribution). A particularly striking feature of these data are additional higher order form factor peaks, relative to what is found in Figure 5.2b, which are quantitatively captured by the model. Moreover, unlike with the pure nanoparticles (Table 5.1), the drug loaded nanogels produce SAXS radii (Figure 5.14b) that are in quantitative agreement with the cryo-TEM results (Table 5.2) for all crosslink densities. A remarkable finding is that the drug loaded nanoparticles are all the same size and considerably smaller than the pure nanogels (Table 5.1). The poor fits at the low q region in Figure 5.15 were due to the existence of large aggregates in addition to the spherical nanoparticles (Figure 5.10).

**Figure 5.14.** (a) SAXS patterns of 10 wt% SDDs of PNIPAm nanogels with 5.0 mol% BIS taken during dissolution in PBS at 22 °C at various time points. The profiles are shifted vertically by factors of 10 for clarity. The solid lines are best fits to sphere model with a Gaussian distribution of radii. (b) Nanoparticle radius and standard deviation (extracted from model fittings) for dissolution of phenytoin-PNIPAm SDDs at all crosslink densities.
Figure 5.15. SAXS patterns of 10 wt% SDD of PNIPAm nanogels with (a) 0.5 and (b) 2.5 mol% BIS taken during dissolution at various time points. The profiles are shifted vertically by factors of 10 for clarity. The solid lines are best fits to a sphere model with a Gaussian distribution of core radii. The nanogel-only sample (9 mg/mL) was fitted to the fuzzy sphere model with a Gaussian distribution of core radii.

Presumably, the spray drying process forces the phenytoin into the nanogels rendering them more hydrophobic than the pure PNIPAm, thus excluding water and eliminating swelling during dissolution. However, there is a limit to the amount of phenytoin that can be taken up by the nanoparticles without compromising the ability to disperse them in PBS. SAXS patterns of 25 wt% SDDs of PNIPAm nanogels taken during dissolution (Figure 5.16) showed no spherical form factors, suggesting that there were few dispersed nanoparticles at this higher drug loading. Upturns in the intensity at low $q$ (i.e., $q < 0.03 \text{ Å}^{-1}$) also suggests the existence of large-sized aggregates (Figure 5.16).
Figure 5.16. SAXS patterns of 25 wt% SDD of PNIPAm nanogels with (a) 0.5, (b) 2.5, and (c) 5.0 mol% BIS taken during dissolution at various time points along with the nanogel-only sample (3 mg/mL). The profiles are shifted vertically by factors of 10 for clarity.

The apparent phenytoin concentration in the dissolution supernatant was measured in PBS with 0.5 wt% fasted simulated intestinal fluid powder (FaSSIF) over the course of 6 h at 22 °C (Figure 5.17). Due to the centrifugation procedure in the *in vitro* dissolution assay, the apparent concentration reflects drugs that are either molecularly dissolved or retained in the supernatant within the nanoparticles (i.e., ≤ 100 nm), which are presumably available for oral absorption. The equilibrium solubility of crystalline phenytoin in PBS/FaSSIF solution at 22 °C (27 ± 1 μg/mL) was previously reported, and is indicated by the dashed line in Figure 5.17. The target phenytoin concentration was 1000 μg/mL; such a high supersaturation results in a strong thermodynamic driving force for drug nucleation and crystal growth.

The SDD dissolution profiles are strongly dependent on the nanogel crosslink density, especially at 10 wt% loading (Figure 5.17a). For a nanogel SDD with 5.0 mol% BIS, phenytoin was rapidly released into the supernatant at a concentration close to the target value and high supersaturation was maintained throughout the 6 h experimental period ($C_{360min} = 936 ± 40 \mu g/mL$). This is consistent with the well-dispersed nanoparticles observed by cryo-TEM over the same period of time (Figure 5.10), from which we conclude that the phenytoin molecules were retained in the supernatant in the form of the
drug-nanogel particles. With 2.5 mol% BIS, the phenytoin concentration increased slowly over time from 280 ± 24 μg/mL at 4 min to 821 ± 11 μg/mL at 360 min. This is attributed to a slower nanogel dissolution process, where the large aggregates (i.e., $R >> 500$ nm) slowly disintegrate into individual dispersed nanoparticles (i.e., $R \approx 43$ nm) during the dissolution experiment (Figure 5.10). With 0.5 mol% BIS, the phenytoin concentration also increased over time, but with a slower rate from 177 ± 15 μg/mL at 4 min to 260 ± 11 μg/mL at 360 min. This also is consistent with the observation of large aggregates (i.e., undissolved SDDs) throughout the 6 h dissolution period (Figure 5.10). Such slowly increasing drug dissolution profiles for nanogels with 0.5 and 2.5 mol% BIS suggest that the nanogel solubility enhancement mechanism is different from the commonly invoked “spring and parachute” model, where drug supersaturation levels decrease with time due to crystallization. Although the large aggregates result in a relatively low supernatant concentration, they remain amorphous as shown by the featureless cryo-TEM electron diffraction patterns (Figure 5.13). At 25 wt% drug loading, the dissolution profiles are approximately the same with little dependence on the nanogel crosslink density (Figure 5.17b), most likely due to the lower nanogel-to-drug ratio. The relatively low phenytoin supernatant concentration during the dissolution of SDD at 25 wt% drug loading is attributed to the existence of large-sized aggregates (i.e., evidenced by the upturn at low $q$ in SAXS data, Figure 5.16), which precipitated out of PBS during dissolution.
Figure 5.17. Dissolution concentration–time curves for SDDs of the indicated PNIPAm nanogels at 22 °C with phenytoin loadings of (a) 10 wt% and (b) 25 wt%. Dashed lines indicate the measured equilibrium solubility of the crystalline drug in PBS/FaSSIF solution at 22 °C.

Effect of the LCST and Behavior of PND Nanogels

The cloud points of the PNIPAm nanogels in PBS were measured by optical transmittance, as shown in Figure 5.18. At all crosslink densities, the PNIPAm nanogel cloud point was approximately 30 °C, which is below physiological temperature (i.e., 37 °C). PNIPAm nanogel SDD dissolution performance was much better at 22 °C (i.e., below the cloud point) than at 37 °C (i.e., above the cloud point), as shown in Figure 5.19.
Figure 5.18. Optical transmittance measurements of PNIPAm and PND nanogels at 0.5 mg/mL in PBS (pH = 6.5) with a heating rate of 0.25 °C/min. All PND nanogels have 5.0 mol% BIS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cloud point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 mol% DMA</td>
<td>34</td>
</tr>
<tr>
<td>32 mol% DMA</td>
<td>37</td>
</tr>
<tr>
<td>42 mol% DMA</td>
<td>41</td>
</tr>
</tbody>
</table>
We have previously shown that PNIPAm-based polymers need to be soluble in aqueous solution to have exhibit favorable drug dissolution performance.\textsuperscript{55} Therefore, a more hydrophilic monomer, DMA, was copolymerized with 5.0 mol% BIS via emulsion polymerization to form poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PND) nanogel particles with a higher LCST. A series of three PND nanogels with increasing DMA composition were synthesized (Figure 5.20), and their corresponding cloud points determined by optical transmittance are summarized in Table 5.4. Incorporating DMA increased the cloud point of the PND nanogel and the cloud points were below, at, and above the physiological temperature for PND nanogels with 21, 32, and 42 mol% DMA, respectively.
Figure 5.20. $^1$H NMR spectra of PND nanogels with 5.0 mol% BIS (after dialysis, lyophilization, drying and re-dissolution in D$_2$O) and pure SDS in D$_2$O.

As shown in Figure 5.21, the hydrodynamic radius distribution and particle density of PND nanogels in PBS at both 22 and 37 °C were measured by DLS and viscometry, respectively. The average $R_h$, $\mu_2/T^2$, and $\rho_{\text{nanogel}}$ values are summarized in Table 5.5. These PND microgels also exhibited narrowly distributions (i.e., low $\mu_2/T^2$ values) in PBS at both temperatures, as shown in Figure 5.21a (note PND with 21 mol% DMA aggregated into large particles at 37 °C). The $R_h$ value increased with increasing DMA composition at constant temperature, indicating that the nanogels were more swollen in PBS with a larger fraction of the more hydrophilic component. Consistent with the DLS results, the nanogel particle density also decreased with increasing DMA composition at constant temperature, due to the nanogel swelling. PND nanogels with both 32 and 42 mol% DMA were more contracted at higher temperature because of the LCST phase behavior, evidenced by the lower $R_h$ and higher $\rho_{\text{nanogel}}$ values at 37 °C than at 22 °C (Table 5.5).
Figure 5.21. (a) Hydrodynamic radius distributions of the PND nanogels at 0.5 mg/mL in PBS solution (pH = 6.5) at 90° scattering angle. (b) Relative viscosity of the P(NIPAm-co-DMA) nanogels in PBS solution (pH = 6.5) as a function of the polymer concentration. Solid lines indicate best fit of the data to Equations 5.7 and 5.8.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_h^{a}$ (nm)</th>
<th>$\mu_2/\Gamma^{2 a}$</th>
<th>$\rho_{\text{nanogel}}^{b}$ (mg/mL)</th>
<th>Sample</th>
<th>$R_h^{a}$ (nm)</th>
<th>$\mu_2/\Gamma^{2 a}$</th>
<th>$\rho_{\text{nanogel}}^{b}$ (mg/mL)</th>
</tr>
</thead>
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<tr>
<td>21 mol% DMA</td>
<td>133</td>
<td>0.03</td>
<td>84 ± 2</td>
<td>32 mol% DMA</td>
<td>174</td>
<td>0.01</td>
<td>70 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123</td>
<td>0.06</td>
<td>209 ± 16</td>
</tr>
<tr>
<td>42 mol% DMA</td>
<td>251</td>
<td>0.04</td>
<td>54 ± 1</td>
<td></td>
<td>217</td>
<td>0.05</td>
<td>108 ± 4</td>
</tr>
</tbody>
</table>

Table 5.5. Characterization of PND nanogels in PBS

Determined by $^a$DLS and $^b$viscometry.

Dissolution of Phenytoin with PND Nanogel SDDs

PND nanogels were spray dried with a 10 wt% loading of phenytoin to produce SDDs. Featureless WAXS patterns confirmed that phenytoin was amorphous in the SDDs (Figure 5.22).
Phenytoin dissolution enhancement at both 22 °C and 37 °C was assessed by the in vitro dissolution assay, and the resulting dissolution profiles are shown in Figure 5.23. At 22 °C, the dissolution performance was the best for the SDD of PND nanogel with 21 mol% DMA, which sustained a high phenytoin supersaturation for 3 h (i.e., $C_{180\text{min}} = 951 \pm 18 \, \mu g/mL$) before a decrease in the phenytoin concentration, presumably due to drug crystallization. With increasing DMA composition in the PND nanogel, the dissolution performance deteriorated (i.e., a more rapid drop of phenytoin supersaturation after the initial burst release, Figure 5.23a). This can be attributed to the lack of drug partitioning into the PND nanogels, especially at higher DMA composition. 2D NOESY spectra for phenytoin and the PND nanogels with varying DMA composition are shown in Figures 5.24-26. For PND nanogels with 21 and 32 mol% DMA, cross peaks were only observed between phenytoin and NIPAm repeat units. No cross peaks between phenytoin and the
DMA repeat units were observed (Figures 5.24-25), suggesting that phenytoin prefers to interact with NIPAm. With 42 mol% DMA, neither phenytoin-NIPAm nor phenytoin-DMA cross peaks were observed in the NOESY spectrum (Figure 5.26), suggesting that the drug partitioning into the nanogel was suppressed at a higher DMA composition.

Figure 5.23. Dissolution concentration–time curves for SDDs of the PND nanogels with 10 wt% phenytoin loadings at (a) 22 °C and (b) 37 °C. Dashed lines indicate the measured equilibrium solubility of the crystalline PTN in PBS/FaSSIF solution at the corresponding temperature.
Figure 5.24. 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 1000 μg/mL of P(NIPAm-co-DMA) nanogel (21 mol% DMA) in D$_2$O. Chemical structures of phenytoin and P(NIPAm-co-DMA) with labeled protons are inserted. Cross peak is circled.

Figure 5.25. 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 1000 μg/mL of P(NIPAm-co-DMA) nanogel (32 mol% DMA) in D$_2$O. Chemical structures of phenytoin and P(NIPAm-co-DMA) with labeled protons are inserted. Cross peak is circled.
Figure 5.26. 2D $^1$H NOESY spectra between 50 μg/mL of phenytoin and 1000 μg/mL of P(NIPAm-co-DMA) nanogel (42 mol% DMA) in D$_2$O. Chemical structures of phenytoin and P(NIPAm-co-DMA) with labeled protons are inserted.

In contrast, at 37 °C, the dissolution performance of the PND nanogel with 42 mol% DMA was significantly improved from 22 °C, as shown by the slower drop of phenytoin supersaturation level (Figure 5.23b). This is correlated with the increase in nanogel particle density from 22 °C to 37 °C (Table 5.5). For PND nanogels with a cloud point either below or at 37 °C (i.e., 21 and 32 mol% DMA, see Figure 5.18 and Table 5.4), the initial phenytoin supersaturation levels were much lower than at 22 °C (Figure 5.23b), possibly due to a rapid supersaturation drop at early time of the dissolution (i.e., < 4 min). In addition, the PND nanogel with 32 mol% DMA had a slightly higher phenytoin supersaturation level than the one with 21 mol% DMA, and for both nanogels, the supersaturation level remained approximately constant towards the end of the dissolution period (i.e., 360 min). When the LCSTs of PND nanogels were above the dissolution
temperature (i.e., 22 °C), dissolution performance was better for the PND nanogels with lower DMA composition (i.e., higher NIPAm composition), presumably because phenytoin partitioned more preferably into nanogels with higher hydrophobicity. In contrast, when the LCSTs of PND nanogels were lower than the dissolution temperature (i.e., 37 °C), dissolution performance was better for the PND nanogels with higher DMA composition, mainly because of the nanogel aggregation. The significantly different dissolution profiles for PND nanogels with 21 and 32 mol% DMA at 22 °C and 37 °C appears to be closely related to the LCST phase behavior of the nanogels, as will be discussed in more detail in the following section.

5.4 Discussion

In this study, the combination of cryo-TEM and SAXS elucidates the hydrophobic drug dissolution and solubility enhancement mechanism with polymer nanogels as a reservoir and delivery vehicle, as illustrated schematically in Figure 5.27. Crosslinked PNIPAm nanogels swell when dissolved in PBS at temperatures below the LCST, or in the good solvent methanol (Figure 5.28). Spray drying a methanol solution of nanogel particles and phenytoin loads the drug molecules into the nanoparticles. Upon dissolution of the SDDs in PBS, the hydrophobic phenytoin molecules are stabilized in the nanogels due to favorable interactions between the drug and polymer as revealed by NOESY (Figure 5.5). As shown in our previous work, a locally concentrated polymer environment such as the corona regions of self-assembled micelles favors drug-polymer partitioning, and therefore, leads to drug solubility enhancement.\textsuperscript{11,13,55} The crosslinked nanogel provides a tunable concentrated region to store and stabilize hydrophobic drug molecules in aqueous solution. Particles loaded with 10 wt% phenytoin are significantly smaller than the pure PNIPAm nanogels in PBS solution (Figure 5.10 and Figure 5.14).
The radius \((R)\) of a dehydrated PNIPAm nanogel can be calculated using the following equation:

\[
\frac{4}{3} \pi R_h \rho_{\text{nanogel}} = \frac{4}{3} \pi R \rho_{\text{bulk}}
\]  

(5.9)

where \(R_h\) is the average hydrodynamic radius of the PNIPAm nanogel in PBS solution from DLS, \(\rho_{\text{nanogel}}\) is the nanogel particle density in PBS solution from viscometry, and an amorphous density of PNIPAm in bulk \(\rho_{\text{bulk}} = 1.03 \text{ g/cm}^3\) was used. In fact, the size of the drug loaded nanoparticles as quantified by SAXS (~45-49 nm, Figure 5.14b) is approximately equal to a completely dry amorphous PNIPAm nanogel plus the additional volume associated with the drug (~43-47 nm, based on Equation 5.9). This indicates that the amorphous drug-nanogel particles are essentially dehydrated due to the hydrophobicity of the drug.

**Figure 5.27.** Illustration of the nanogel sequestration mechanism to store and stabilize hydrophobic drug molecules.
Figure 5.28. Hydrodynamic radius distributions of the PNIPAm nanogels at 1 mg/mL in MeOH at 22 °C at 90° scattering angle.

The *in vitro* dissolution performance was better for SDDs using PNIPAm nanogels with a higher crosslink density, especially at 10 wt% drug loading (Figure 5.17). At the highest crosslink density (i.e., 5.0 mol% BIS), the SDDs rapidly disintegrated into well-dispersed spherical nanoparticles (~44 nm) in PBS, as revealed by cryo-TEM images (Figure 5.10). With a relatively low crosslink density (i.e., 0.5 and 2.5 mol% BIS), large (>500 nm) aggregates comprising the smaller nanoparticles were observed to persist after mixing SDDs in PBS (Figure 5.10). We attribute this behavior to interpenetration and entanglement of dangling chains at the nanogel particle surface.\(^{217,218}\) Such chain penetration is most likely to occur at higher volume fractions,\(^{130}\) as occurs during the spray drying process with the rapid evaporation of the methanol.\(^{82}\) With a higher NIPAm-to-BIS ratio at low crosslink density (e.g., ~200 at 0.5 mol% BIS), the dangling chains at the nanogel surfaces are more likely to interpenetrate and entangle other particles leading to the formation of large scale aggregates that persist after dissolution. SDDs formed from pure nanogels, i.e., devoid of hydrophobic drug molecules, completely disperse in
PBS immediately after vortexing, resulting in a homogeneous solution as documented by DLS, regardless of the crosslink density (Figure 5.12). Addition of hydrophobic drug molecules to the nanogel particles influences both the rate of dissolution of the SDDs and the size of the constituent nanoparticles following dispersion in an aqueous medium. We infer that dangling chains from one particle that interpenetrate another effectively crosslink them together. Favorable interactions between the phenytoin and polymer chains highlighted in Figure 5.5 will contribute to stabilizing these aggregates by restricting the ability of water to swell and eventually hydrate the peripheral chains, which is required to achieve full dispersion. Cryo-TEM (Figure 5.10) and SAXS (Figure 5.14) confirm that those particles that are released from the SDD are of a reduced size consistent with loading of phenytoin. Absence of cryo-TEM diffraction in the residual SDD aggregates during dissolution confirms that crystallization is not responsible for the lack of particle dispersion. Taken together, this evidence supports the dangling chain mechanism. Higher degrees of crosslinking should lead to fewer and shorter dangling chains, hence less of a tendency for particles to get stuck together, consistent with what is seen in Figure 5.10. Friesen et al. have noted that drug/polymer nanostructures are critical for enhanced oral absorption.²⁴ We believe the design principles established here make nanogels effective reservoirs for solubilizing hydrophobic drugs for oral administration.

Taken together, Figure 5.18 and Figure 5.23 clearly demonstrate that the LCST plays an important role in determining the drug dissolution profile. A delicate balance between aqueous solubility and hydrophobicity of the polymer, and drug, establishes the viability of delivering a hydrophobic drug in an oral dosage form using a polymer excipient.⁵⁵,¹⁰⁰ The polymer needs to remain soluble in aqueous solution at physiological temperature (i.e., 37 °C). The conformational change from extended coils to contracted globules for PNIPAm at approximately 32 °C upon heating (i.e., LCST), will shift this balance of interactions at physiological temperature, which can result in poor drug oral
bioavailability. Approaches to rectifying this problem include blending PNIPAm with other polymers (e.g., HPMCAS), reducing molar mass (e.g., \( \lesssim 4 \text{ kg/mol} \)), or co-polymerizing with more hydrophilic monomers. The latter two strategies essentially increase the LCST of PNIPAm. On the other hand, hydrophobic polymers are more likely to interact with hydrophobic drug molecules. For instance, on the basis of the NOESY results (Figures 5.24-26), phenytoin interacts more preferably with NIPAm than DMA repeat units in the nanogel. The nanogel LCST was tuned by randomly incorporating DMA in the network chains at various compositions to slightly increase the hydrophobic environment that favors drug-nanogel partitioning, while retaining the ability to disperse the nanoparticles in aqueous solution at physiological temperature.

The mechanism of sequestering hydrophobic drug molecules in a chemically crosslinked nanogel system has been elucidated by this work. Hydrophobic drugs are typically loaded in the relatively more hydrophobic core part of surfactants and micelles for enhanced drug solubility. Our strategy is distinctly different from the core-loading method, and requires no extra triggering mechanism to release the entrapped drugs in the micelle core. Phenytoin molecules were partitioned into polymer nanogels, which can serve as a reservoir to maintain high drug supersaturation while retarding drug crystallization. The drug dissolution and solubility enhancement mechanism discovered in this study is somewhat different than the commonly recognized “spring and parachute” approach, where amorphous drugs are often rapidly released upon dissolution and the subsequent drop of supersaturation is caused by drug crystallization. At lower nanogel crosslink density, the “spring” effect of the amorphous drug is dramatically delayed due to nanoparticle agglomeration. The fact that such delayed drug release (i.e., “spring” effect) could be tuned by the crosslink density makes nanogel promising carriers for controlled drug release applications. The nanogel crosslink density, LCST, and balance between hydrophilic and hydrophobic comonomers are all important
parameters to be optimized for ideal hydrophobic drug solubility and dissolution enhancement in an oral therapeutic method.

5.5 Conclusions

We have investigated the dissolution mechanism of PNIPAm based nanogels, formulated as SDDs, with a rapidly crystallizing model drug phenytoin. Based on DLS and viscometry experiments, pure PNIPAm nanogels are more contracted (i.e., smaller $R_h$ and higher $\rho_{\text{nanogel}}$) in PBS at a higher crosslink density (i.e., mol% BIS). Phenytoin-nanogel spatial proximity in aqueous solution was revealed by NOESY. In vitro dissolution profiles of the SDDs were highly dependent on the nanogel crosslink density. An approximately full release of phenytoin was achieved and maintained throughout a 6 h experiment period at 22 °C by using SDDs of PNIPAm nanogel with 5.0 mol% BIS at 10 wt% drug loading. At lower crosslink density, the phenytoin concentration slowly increased over time as the SDDs disintegrated releasing drug loaded nanogel particles. Using a combination of cryo-TEM and SAXS experiments, the phenytoin solubility during dissolution was found to be directly correlated with the existence of drug-laden nanogel particles (i.e., $R \approx 43$ nm). Large nanoparticle aggregates (i.e., $R >> 500$ nm) persist for long times during dissolution of SDDs formed by lower crosslink density nanogels, which is explained based on particle-particle adhesion due to cross-particle penetration of dangling chains.

The effects of an LCST on drug-nanogel interactions and the subsequent in vitro dissolution performance were systematically investigated using particles prepared with NIPAm and DMA monomers, referred to as PND nanogels. Phenytoin molecules interact more preferably with NIPAm than DMA, and therefore, dissolution performance was better (i.e., higher drug release and longer supersaturation maintenance) for PND
nanogels with a higher NIPAm composition at temperatures below the LCST. However, sufficient DMA needs to be incorporated into the nanogel to make it soluble at physiological temperature (i.e., the nanogel LCST needs to be slightly above 37 °C) for oral drug delivery applications. Thus, the delicate hydrophobicity–hydrophilicity balance of the nanogel is crucial when designing an optimal nanogel for hydrophobic drug solubility enhancement. This mechanism and structure-property relationship discovered in this study provides a promising direction for the rational design of polymer nanogels as effective excipients to solubilize other intractable hydrophobic drugs.
6. Concluding Remarks

6.1 Summary

Three different polymer systems were investigated in this dissertation for oral drug delivery applications, including a blend of HPMCAS and self-assembled PNIPAm micelles in Chapter 3, PND-b-PS diblock polymer micelles in Chapter 4, and crosslinked PNIPAm nanogels in Chapter 5. The discoveries in all these three chapters can be distilled into a single underlying mechanism: a locally concentrated polymer environment can be achieved either physically (i.e., micelle self-assembly) or chemically (i.e., crosslinking); the crowding of the polymer chains can facilitate the partitioning of the hydrophobic drugs, and therefore maintain a high drug supersaturation by inhibiting drug crystallization.

In Chapter 3, blends of HPMCAS and PNIPAm homopolymers were used to deliver a model drug phenytoin in the form of spray-dried dispersions. We showed that dodecyl (C\textsubscript{12})-tailed PNIPAm can self-assemble into narrowly distributed micelles with C\textsubscript{12} cores in phosphate-buffered saline (PBS, pH=6.5). A synergistic effect of the polymer blends was observed during the in vitro dissolution of the phenytoin-loaded spray-dried dispersions, where the blend outperformed both individual polymers at the same phenytoin loading. Presumably the HPMCAS mainly facilitates the rapid dissolution of phenytoin (the so called “spring” effect) and the self-assembled PNIPAm maintains the phenytoin supersaturation by inhibiting both drug nucleation and crystal growth (the “parachute”). To elucidate the mechanism, we found that phenytoin interacts more preferably with PNIPAm than HPMCAS in aqueous solution. Furthermore, the partitioning of phenytoin to PNIPAm is stronger for the self-assembled micelles with a higher corona density. Thus, the corona region of the micelle can serve as a reservoir to sequester hydrophobic drug molecules at a supersaturated concentration. We hypothesize
that the polymer blend strategy, coupled with the micelle corona sequestration method, can be applied universally to other hydrophobic drug molecules.

In Chapter 4, a series of PND-b-PS diblock polymers, with the same corona block length but varying core block length, were used as model micellar systems to further investigate the micelle corona loading mechanism. We found that the micelle corona density increased as the core block length increased because a higher number of polymer chains were incorporated into a single micelle core with a longer core block. Two hydrophobic model drugs, phenytoin and nilutamide, were investigated in this study. In both cases, the drug partitioning strength to the polymer micelle increased as the corona density of the diblock polymer micelle increased. This trend is consistent with what we discovered in Chapter 3. It is worth noting that the rapid dissolution and full release of phenytoin can be achieved in vitro by using only the self-assembled micelles in this chapter. The LCST of the PND corona block was tuned to be slightly higher than the physiological temperature. It is important to have a corona block that is slightly hydrophobic for preferable drug partitioning, but also soluble in aqueous solution at physiological temperature. Thus, the delicate balance between the hydrophobicity and hydrophilicity (i.e., LCST) is a key parameter for designing an effective micelle corona reservoir.

In Chapter 5, we extended the polymer crowding mechanism to a chemically crosslinked nanogel system. A series of PNIPAm nanogels with varying crosslink density were used as model systems to investigate the crowding effect on model drug phenytoin dissolution. We found that the phenytoin in vitro dissolution performance was the best for PNIPAm nanogels with the highest crosslink density. A combination of cryo-TEM and SAXS confirmed that phenytoin was loaded and stabilized in the nanogels, by forming drug/polymer nanoparticles with smaller sizes than the pure nanogels in aqueous solution. The dissolution profiles of the spray-dried dispersions were directly correlated with the
nanoparticle evolution. When using nanogels with lower crosslink density, nanoparticle aggregates persisted for a long time due to cross-particle entanglement of the nanogel dangling chains, resulting in lower phenytoin apparent concentration in vitro. In addition, consistent with our findings for the micellar systems in Chapter 4, the LCST of the nanogels have an important influence on the drug dissolution profiles of the corresponding ASDs.

6.2 Future Research Directions

In the final section, possible future research directions will be discussed based on what has been learned from this dissertation. The future directions include: 1. the relatively new characterization techniques developed in this dissertation can be potentially applied to other drug/polymer ASD systems; 2. the underlying mechanism derived from Chapters 3-5 can be used to design other effective polymer materials to solubilize hydrophobic model drugs.

First, a combination of cyro-TEM and SAXS can be used to not only directly visualize the potential drug/polymer nanoparticles in aqueous solution, but also to quantify the particle size in situ during the in vitro dissolution test. What we learned from Chapters 3 and 5 is that a strong correlation exists between the hydrophobic drug dissolution profile and the drug/polymer nanoparticle evolution over time. For model drug phenytoin, such correlation exists for two different polymer systems: micelles and nanogels. Meanwhile, recent work by Ricarte et al. demonstrated that the dissolution profiles of both phenytoin and probucol were also highly dependent on the drug/polymer nanoparticle evolution, using the same polymer HPMCAS. Therefore, it is reasonable to verify that the drug/polymer nanoparticle evolution concept can be universally applied to other polymer/drug systems. The first drug/polymer system that can be used for potential
nanoparticle investigation in the near future is the one described in Chapter 4. We’ve observed different drug dissolution profiles for two model drugs, phenytoin and nilutamide, by using a series of micelle-forming diblock polymers. As shown in Figure 6.1, preliminary SAXS scattering patterns of the ASDs showed shoulders that were presumably caused by some particle form factors (i.e., $q = 1-3 \times 10^{-2} \text{ Å}$), while scattering pattern of the pure polymer had much less intensity at the same $q$ region. This suggests that nanoparticles exist during the dissolution of phenytoin/PND$_{34}$-C$_{12}$ ASDs for the entire 6 h period. This SAXS patterns is highly correlated with the dissolution profile shown in Figure 4.20 in Chapter 4, suggesting the phenytoin was supersaturated in the form of drug/polymer nanoparticles. Cryo-TEM can be used to directly visualize the nanoparticles. Future work could extend such investigation of the drug/polymer nanoparticles to different model drugs and polymer nanostructures.

![Figure 6.1. SAXS patterns of SDDs of phenytoin and PND$_{34}$-C$_{12}$ at 10 wt% loading taken during dissolution in PBS at 37 °C at various time points. The profiles are shifted vertically by factors of 10 for clarity. Pure PND$_{34}$-C$_{12}$ in PBS at 37 °C at 9 mg/mL is also plotted.](image)

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In Chapter 5, electron diffraction patterns of the nanoparticles can determine whether these structures are amorphous or crystalline in aqueous solution. It has been observed in multiple polymer systems that the drug concentration was lower than the target one during \textit{in vitro} dissolution of ASDs (i.e., Scenario (c) in Figure 1.1), especially with higher drug loadings.\textsuperscript{11,53,55} One remaining question is whether the low drug concentrations were caused by drug crystallization or aggregation of the amorphous drug/polymer particles. The \textit{in vitro} microcentrifuge dissolution test is based on the assumption that the drugs in the supernatant after centrifugation are the ones available for oral drug delivery. However, we’ve shown in Chapter 5 that relatively low drug apparent concentrations \textit{in vitro} during the microcentrifuge dissolution test can be caused by large drug/polymer aggregates; and drugs in these large aggregates remained amorphous. Possibly the drug/polymer nanoparticles are too hydrophobic to be soluble in aqueous solution even without drug crystallization, especially at higher drug loadings. Electron diffraction pattern of the drug/polymer particles during cryo-TEM imaging will unambiguously determine their amorphous or crystalline nature. One limitation of using electron diffraction during cryo-TEM is that large drug/polymer particles may be too big (e.g., micron-sized) to be captured in a TEM grid since vitrified ice thickness is typically on the order of 100 nm.\textsuperscript{224} As an alternative, synchrotron-sourced WAXS can be used to determine whether the centrifuged component during the dissolution test are crystalline or amorphous. As discussed in Chapter 1, the fast acquisition time and high sensitivity makes synchrotron-sourced X-ray experiments ideal to monitor the samples \textit{in situ} during dissolution tests. We also learned from Chapters 3-5 that how close the dissolution temperature to the LCST of the polymer will significantly change the drug \textit{in vitro} dissolution profiles. We conclude that the LCST of the polymer should be tuned to be slightly higher than the physiological temperature for both the strong drug partitioning and aqueous solubility. The NOESY and PFG-NMR/DOSY experiments developed in
this dissertation can be used to quantify the drug partitioning strength, as a function of temperature, to study the LCST and chemical structure effect. At higher temperatures, temperature gradients in the NMR sample can cause the convection, which will result in inaccurate diffusion measurement. Thus, DOSY pulse sequences with convection compensation incorporated should be used to avoid this problem.

Second, the mechanism of drug sequestration in the locally concentrated polymer environment, demonstrated by various polymer systems in Chapters 3-5, can be potentially generalized to other polymer structures. In addition to the micelles and nanogels covered in this dissertation, there are a variety of other polymer structures that can facilitate the crowding of the repeat units, such as star polymers, graft polymers, and dendrimers. In Chapters 3 and 4, we showed that dodecyl-tailed NIPAm containing polymers (i.e., either homopolymers or statistical copolymers) can self-assemble into micelles with the dodecyl core; and the micelle corona region can serve as a reservoir to facilitate the drug partitioning. The structures and properties of these self-assembled micelles are very similar to star polymers. In fact, a star polymer form factor was used to describe the SAXS pattern of the self-assembled PND-C$_{12}$ micelle in aqueous solution, as shown in Figure 4.3 in Chapter 4. Plummer et al. have synthesized 4-armed PNIPAm by RAFT polymerization and showed that the chain density depends on the chain molecular weight and temperature relative to the LCST. Schramm et al. have obtained 4–12-armed star polymers with poly(ethylene glycol) methacrylate shell and poly($\varepsilon$-caprolactone) core by a combination of ring-opening polymerization and atom transfer radical polymerization. The number of arms is expected to change the local crowding of the polymer chains, and therefore dictate the drug partitioning strength and the subsequent dissolution performance. Graft polymers have been extensively studied for drug delivery applications, especially by using thermoresponsive grafts. The graft density can be controlled by using two comonomers with different compositions, and
its correlation with the drug partitioning strength can be systemically investigated by a series of model graft polymers with various graft densities. In addition, it has been shown that PNIPAm-functionalized dendrimers can also be used as nanocarriers to deliver a water insoluble bioactive compound malloapelta B. The number of generations of a dendrimer can be varied to study the chain crowding effect. Last, the nanogel platform showcased in Chapter 5 offers versatile processing possibilities of simultaneous drug loading during spray drying. Amphiphilic block copolymers (e.g., PNIPAm-b-PEO) can be included during the spray drying process to coat the drug loaded nanoparticles, thus facilitating the particle dispersion even in the low nanogel crosslink density limit. With judicious design of the block copolymer coatings, controlled release triggered by pH change from stomach to small intestine environment or timed polymer degradation might be realized to further benefit bioavailability in the future.

These abovementioned model polymer systems can be used to verify whether we can apply and generalize the drug sequestration mechanism developed in this dissertation to other polymer materials. With the three different polymer systems showcased in this dissertation, we hope to bridge the gap between the fundamental understanding of polymer science and the development of new pharmaceutical formulations. The polymer structure-property relationships discussed in this dissertation should inform the design and optimize ASD formulations for oral drug delivery application. A better understanding of the underlying drug solubility enhancement mechanism is essential to the pharmaceutical development, and can ultimately provide more effective polymer-based products.
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