RATIONAL DESIGN of DRUG FORMULATIONS USING
COMPUTATIONAL APPROACHES

BY

Loan Kim Huynh

Supervisor: Dr. Christine Allen
Co-supervisor: Dr. Régis Pomès

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University of Toronto

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Abstract

Theory has been used to complement experiment in the development of both drugs and delivery systems. Theoretical methods are capable of identifying the molecular basis of drug formulation inadequacies and systematic theoretical studies may suggest fruitful avenues for material modification. This thesis highlights the utility of computer-based theoretical calculations for guiding the design of drug formulations and enhancing material-drug compatibility and stability. Specifically, the present work explores the applications of semi-empirical methods and atomistic molecular dynamics (MD) simulations to enhance the performance of nano-emulsions and polymer micelle formulations for the delivery of hydrophobic drugs. This work includes three separate studies preceded by an introductory summary of available theoretical techniques.

The first study evaluates the accuracy and reliability of semi-empirical methods and MD simulations as means to select suitable excipients to formulate the anti-cancer drug docetaxel in an emulsion. Here, simulations accurately predict the rank order of drug solubility in various excipients, suggesting that simulation is useful for library enrichment.
In the second study, a drug conjugation approach is used to further improve the stability and solubility of docetaxel in a triglyceride-based nano-emulsion. Here, optimal conjugates are identified with computer-based theoretical calculations and conjugates with formulation-compatible moieties are synthesized. As predicted, the conjugates exhibit enhanced solubility and loading efficiency in a nano-emulsion.

The goal of the third study is to rationally design a stable unimolecular star copolymer that, as a unimer, does not disassemble upon the dilution that accompanies intravenous injection. Here, MD simulation is used to systematically investigate the solution properties of differently composed star copolymers. Overall, star copolymers with a hydrophobic PCL core ≤ 2 kDa and hydrophilic PEG blocks approaching 14.6 kDa per arm are predicted to form unimolecular micelles that remain unimeric at high concentrations.

The studies presented in this thesis demonstrate that theoretical approaches are useful for fast pre-screening of drug formulation materials and for the development of delivery systems and drug derivatives.
Dedicated to my parents, my sisters and brother, and my husband
Acknowledgements

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<th>Description</th>
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<tr>
<td>CED</td>
<td>cohesive energy density</td>
</tr>
<tr>
<td>CG</td>
<td>coarse-grain</td>
</tr>
<tr>
<td>CMC</td>
<td>critical micelle concentration</td>
</tr>
<tr>
<td>COM</td>
<td>center of mass</td>
</tr>
<tr>
<td>COMPASS</td>
<td>Condensed-phase Optimized Molecular Potentials</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DTX</td>
<td>docetaxel</td>
</tr>
<tr>
<td>DPD</td>
<td>dissipative participle dynamics</td>
</tr>
<tr>
<td>EE</td>
<td>entrapment efficiency</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>FH</td>
<td>Flory–Huggins</td>
</tr>
<tr>
<td>fs</td>
<td>femto-second</td>
</tr>
<tr>
<td>IC50</td>
<td>inhibitory concentration</td>
</tr>
<tr>
<td>GCM</td>
<td>group contribution methods</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>MC</td>
<td>Monte Carlo</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>MePEG</td>
<td>methoxy poly(ethylene glycol)</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NE</td>
<td>nano-emulsion</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NVT</td>
<td>constant-temperature, constant-volume ensemble</td>
</tr>
<tr>
<td>OPLS</td>
<td>optimized potentials for liquid simulations</td>
</tr>
<tr>
<td>PAMAM</td>
<td>polyamidoamine</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PLA</td>
<td>poly (d,l-lactide)</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>ps</td>
<td>pico-second</td>
</tr>
<tr>
<td>PBLG</td>
<td>poly(γ-benzyl L-glutamate)</td>
</tr>
<tr>
<td>PBCL</td>
<td>poly(α-benzylcarboxylate-ε-caprolactone)</td>
</tr>
<tr>
<td>PChCL</td>
<td>poly(α-cholesteryl carboxylate-ε-caprolactone)</td>
</tr>
<tr>
<td>PFOB</td>
<td>perfluorooctylbromide</td>
</tr>
<tr>
<td>PTX</td>
<td>paclitaxel</td>
</tr>
<tr>
<td>POPC</td>
<td>palmitoyloleoylphosphatidylcholine</td>
</tr>
<tr>
<td>RDF</td>
<td>radial distribution function</td>
</tr>
<tr>
<td>Rg</td>
<td>radius of gyration</td>
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<tr>
<td>SPC</td>
<td>simple point charge</td>
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<tr>
<td>SCPs</td>
<td>star shape diblock copolymers</td>
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<tr>
<td>SP</td>
<td>solubility parameter</td>
</tr>
<tr>
<td>TMC</td>
<td>trimethylene carbonate</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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Chapter 1: Computational Approaches to the Rational Design of Nanotechnologies as Drug Delivery Systems

Abstract

Recent advances in molecular simulation methodologies and computational performance may be used to guide the rational design and optimization of nanotechnology delivery systems. Nevertheless, methods that predate computers remain important tools in this domain. This chapter begins with a brief overview of available theoretical approaches to the development of contemporary drug delivery systems. These approaches include solubility parameters, Flory-Huggins theory, analytical predictions of partition coefficients, and molecular simulations. These methods are then compared as they relate to the optimization of drug-material pairs using important performance-related parameters including the size of the delivery particles, their surface properties, and the compatibility of the materials with the drug to be sequestered. Next, this chapter explores contemporary efforts to optimize a representative selection of existing nanotechnologies, including nanoemulsions, linear and star block copolymer micelles, and dendrimers. The chapter concludes with an outlook on the challenges remaining in the successful application of these theoretical methods to the development of new drug formulations. Finally, the research objectives of this thesis are presented in the last section.
1.1. Nanotechnologies as Drug Delivery Systems

It is estimated that 40% of the small molecules that are emerging as new drug candidates are hydrophobic. Full exploitation of the therapeutic potential of these drugs relies on their solubilization in non-toxic, biocompatible and/or biodegradable formulations that solubilize, protect, and transport drug molecules, finally releasing drug at the target tissue. This chapter focuses on efforts to optimize nanotechnology delivery materials and drug solubility in these materials.

The last few decades have produced an astounding number of nanotechnologies; systems comprising particles with at least one dimension between 1 and 100 nm. Nanotechnologies share this size range with many biologically-relevant molecules, and biomedical applications of nanotechnologies abound. Among these, nanoparticles composed of organic molecules are capable of modifying the apparent physicochemical properties of sequestered compounds. For example, drugs encapsulated by nanotechnologies exhibit enhanced aqueous solubility, and can be targeted to diseased tissue, improving the drug’s therapeutic index. Several drugs relying on formulation in advanced delivery systems have been approved for human use while others are in late-stage preclinical or clinical development.

This chapter highlights theoretical approaches that are available to predict solubilities and miscibilities and thereby assist in the rational design of contemporary nanotechnology platforms. These approaches are discussed in application to nanotechnology platforms including nanoemulsions, polymeric micelles, liposomes, and dendrimers with hydrophobic cores and hydrophilic peripheries (Scheme 1.1). Theoretical studies of liposomes are reviewed elsewhere.
Scheme 1.1: A variety of nanotechnology platforms that have been investigated using molecular simulations. (a) Nano-emulsions with an oily hydrophobic core and hydrophilic surfactant corona. (b) Linear or star shaped amphiphilic block copolymer micelles composed of hydrophobic and hydrophilic blocks. The ends of the hydrophobic blocks are covalently bonded for the star shaped block copolymer micelles. (c) Dendrimers with a hydrophobic core and hydrophilic peripheries. (d) Liposomes (not covered in this chapter) have a lipid bilayer that forms a hydrophobic domain for solubilizing hydrophobic drugs, and an inner aqueous volume for solubilizing hydrophilic drugs. Stealth liposomes are further coated with PEG. In the above platforms, (a-d) the hydrophobic core (pink) serves as cargo space for lipophilic solutes and the hydrophilic shell (blue), often composed of PEG, protects the core from the aqueous environment. The hydrophilic headgroups of surfactants are shown in teal.
For a given application, the most appropriate nanotechnology platform is rarely obvious. Further, within each platform, nanotechnologies must be optimized to achieve the desired properties. These properties are predictable by theoretical approaches, which may be employed to accelerate development efforts. Important performance related parameters of drug delivery systems include the compatibility between the drug and the materials of the delivery system, and the surface properties and size of the particles.\textsuperscript{12, 15} Optimization of the compatibility between the drug and the solubilizing media has been shown to result in significant improvements in drug loading, drug retention, and thereby the chemical stability of the formulation.\textsuperscript{8-10} Additionally, the surface properties of the delivery system influence its stability during storage and following \textit{in vivo} administration.\textsuperscript{25, 26} Delivery systems with a non-ionic, sterically stabilized, hydrophilic surface are less susceptible to adsorption of plasma macromolecules\textsuperscript{11, 12, 27} and clearance from the blood stream by the mononuclear phagocytic system.\textsuperscript{12, 15} Steric stabilization of nanoparticles may be achieved by coating their surface with specific polymers.\textsuperscript{12} In this connection, the most widely used shell-forming material is poly(ethylene glycol) (PEG), a unique polymer with remarkable flexibility owing to its high aqueous solubility.\textsuperscript{28-34}

1.2. Theoretical Approaches to Developing Nanotechnologies

Theory has been used to complement experiment in the development of effector molecules such as drugs.\textsuperscript{24, 35-37} Whereas experimental methods are highly efficient in lead drug identification and optimization,\textsuperscript{38} high-throughput experimental screening methods that employ heterogeneous mixtures are less amenable to the development of drug formulations. While it remains viable to experimentally prepare and separately
evaluate a variety of drug formulations, this is costly and time consuming.\textsuperscript{22} Further, while high-throughput experimental material selection appears to be feasible in some cases,\textsuperscript{39} material optimization involves significantly more possibilities and the number of full factorial combinations quickly becomes unfeasibly large for experimental methods.\textsuperscript{39} More fundamentally, experimental studies of novel drug formulations do not disclose the molecular basis for their performance (e.g. the reason why the drug precipitates), and hence do not indicate possible avenues by which they may be improved. In contrast, theoretical methods are capable of identifying the molecular basis of drug formulation inadequacies\textsuperscript{21, 35} and systematic theoretical studies may suggest fruitful avenues for material modification.\textsuperscript{24, 35, 40}

Theoretical calculations of the physicochemical properties of liquid mixtures have been applied for more than a century. In 1898, Bodländer predicted the aqueous solubility of salts based on the electrode potentials of the ions and their heats of solidification.\textsuperscript{41, 42} In 1931, Scatchard\textsuperscript{42} introduced the first solubility parameter (SP), a general theoretical measure of miscibility. In 1950, Hildebrand\textsuperscript{43} extended the utility of SPs with a group contribution method that evaluates complex molecules as the sum of their parts. In 1942, the liquid lattice theories of Flory\textsuperscript{44, 45} and Huggins\textsuperscript{44} were merged to generate the Flory-Huggins interaction parameter that, different from the SP, directly evaluates the miscibility of two compounds. In 1974, Nys and Rekker predicted lipophilicity of small molecules.\textsuperscript{46} More recently, the advent of powerful computers has allowed the automation of these methods and the introduction of new ones based on molecular simulations.\textsuperscript{47}
Theoretical approaches constitute an important component of the development and selection of drug delivery systems in part because of the large number of drug candidates that require formulation and the numerous strategies that may be pursued.\textsuperscript{1} A key requirement for the successful chemical design of nanotechnologies as drug delivery systems is an accurate understanding of the molecular properties associated with the performance of the formulations. A variety of computational methods exist to predict physicochemical properties including the solubility and lipophilicity of small molecules\textsuperscript{48, 49} and structural properties of formulation materials (Scheme 1.2).\textsuperscript{40, 50-53} These theoretical approaches can be broadly divided into two categories: analytical models and molecular simulations.
Scheme 1.2: A variety of computational methods exist to predict various physicochemical properties and structural properties of formulation materials.
1.2.1. Analytical models

Phenomenological models are presumptive relationships expressed in a convenient mathematical form and parameterized to reproduce known quantities. Included in this category are analytical methods,\(^{38,54-56}\) which are based on experimental data. There are, for example, a variety of theories that apply a group contribution method to sum structure-based molecular descriptors over predefined fragments of a complex molecule. These methods are widely used because they predict relevant pharmaceutical properties, such as lipophilicity\(^ {49}\) and solubility parameter\(^ {48,57,58}\) very quickly. For example, the lipophilicity or SP of thousands of compounds can be computed overnight using computerized automation of these methods. Applications include predicting which drug-material pairs will perform optimally as a formulation based on the theoretical cohesive energy of the compounds,\(^ {48}\) or predicting the loading and/or retention of drug in the formulation.\(^ {49}\)

It has been estimated that the chemical space of small drug-like molecules includes in excess of \(10^{60}\) compounds.\(^ {36}\) Synthesis and systematic characterization of these molecules is costly and time consuming because they are so numerous in comparison to \textit{bona-fide} drugs. For this reason, theoretical methods have been widely used as complementary tools in many stages of drug development including drug discovery and evaluation of drug activity,\(^ {35-37}\) development of drug formulations,\(^ {59}\) and studies conducted \textit{in vitro}.\(^ {38,56}\) Particularly, analytical approaches using computerized automation have become essential research tools for the prediction of the absorption, distribution, metabolism, excretion and toxicity of drug candidates, especially in early stages of drug design and lead compound refinement.\(^ {24,35}\) As reviewed by Lipinski \textit{et al}...
high-throughput screening using experimental and computational approaches have successfully accelerated the identification and evaluation of drug-like molecules. In 1989, the calculation of physicochemical properties was first automated by Pfizer laboratories facilitating high throughput screening of drug-like compounds.\textsuperscript{38}

The utility of analytical models will be discussed throughout this chapter. In general, they are excellent tools to rapidly eliminate material combinations that are unlikely to be miscible and thus enrich the probability of compatibility within the remaining materials (library enrichment). These methods are, however, often quantitatively wrong and are most useful as preliminary tools to reduce the number of evaluations to be conducted with more time-consuming theoretical or experimental approaches.

\subsection{1.2.2. Molecular simulations}

Physics-based models generate predictions from first principles, or approximations thereof.\textsuperscript{38, 54-56} The simplest of these are closed-form approximations that have analytical solutions. These are, however, unavailable for molecules of interest to pharmaceutical formulation due to the complex nature of their conformational and chemical properties. In their place, one may use molecular simulations\textsuperscript{47, 60} to solve \textit{n}-body problems based on the application of statistical mechanics to quantum- or molecular-mechanics force fields. Methods employing molecular mechanics use simplifying assumptions to reduce computational requirements and are thus semi-empirical in nature. These simulations may be conducted \textit{via} Monte Carlo (MC) or molecular dynamics (MD) approaches. MC simulations employ a stochastic algorithm to
accept or reject arbitrary configurational moves by evaluating the change in potential energy, whereas MD simulations numerically integrate the differential equations of motion to generate a time-trajectory.\textsuperscript{47, 60}

Simulations can provide quantitative measurements at the atomistic level that are difficult to obtain experimentally.\textsuperscript{40, 50, 61} For example, simulations can predict nanoparticle size and shape,\textsuperscript{40, 50, 61, 62} which are key modulators of biodistribution and clearance.\textsuperscript{21, 63-65} Simulations can also predict the hydrated vs. solvent-protected surface area, a factor that influences stability.\textsuperscript{40} The structural and dynamic properties of nanotechnologies and their cargo can be characterized by using MC \textsuperscript{64-67} and MD\textsuperscript{40, 50, 62, 63, 68} simulations. Simulations can predict the loading and release of drug from a formulation, either directly,\textsuperscript{21, 63, 69, 70} or by predicting the compatibility between the drug and delivery materials.\textsuperscript{40, 48, 64, 71, 72} Simulations can be applied to correlate drug release with kinetic properties, such as diffusion,\textsuperscript{21, 69} thermodynamic properties, such as orientational preferences and free volumes,\textsuperscript{62, 67, 73} and compositional properties, such as the nature and molecular weight (MW) of hydrophobic/hydrophilic blocks of a copolymer.\textsuperscript{40, 74} Importantly, kinetic properties are unavailable to analytical thermodynamic methods. Finally, simulations of cell membrane mimetics can predict drug and nanoparticle permeabilities across biomembranes.\textsuperscript{21, 75}

1.3. Identifying Optimal Drug-Material Pairs

During formulation development, promising drug-material pairs can be identified by predicting the strength of drug-material interactions through the estimation of parameters such as solubility, lipophilicity, and chemical compatibility.
The early parts of this section review analytical approaches that are available to evaluate drug-material pairs: solubility and SPs in section 1.3.1, Flory-Huggins theory in section 1.3.2, and lipophilicity and log P in section 1.3.3. Section 1.3.4 then introduces molecular simulation approaches. Theoretical identifications of prodrugs with enhanced solubilities are reviewed in section 1.3.6 and, finally, section 1.3.7 discusses attempts to predict drug loading and retention within formulations.

1.3.1. Solubility and solubility parameters

The aqueous solubility of a drug strongly influences its biological activity and can be predicted using various computational models. Solubility is a measure of the maximum amount of solute that forms a homogeneous solution with a specified solvent under equilibrium conditions. In contrast, a solubility parameter (SP) is a scalar value that gives an indication of the predicted miscibility of two components. Materials with similar SPs are indicated to be miscible. SPs can be calculated very quickly by group contribution methods and are useful for ranking materials based on their relative predicted abilities to solubilize a drug. In comparison to molecular simulation or experimental approaches, calculation of SPs using group contribution methods are fast because they avoid evaluation of different materials as a mixture (e.g. drug A in oil B), as would be necessary in a direct evaluation of solubilities.

Hildebrand and Hansen approaches have been used to calculate SPs as shown in Equations 1a and b, respectively, in Table 1.1, and reviewed elsewhere.
Table 1.1: Theoretical methods used for the calculation of physical-chemical properties of compounds

<table>
<thead>
<tr>
<th>Theoretical relations</th>
<th>Component symbols</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta_{\text{HIL}} = \sqrt{\frac{E_{\text{coh}}}{V}} = \sqrt{\text{CED}}$</td>
<td>$\delta_{\text{HIL}}$ – Hildebrand solubility parameter</td>
<td>79</td>
</tr>
<tr>
<td>$E_{\text{coh}}$ – cohesive energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$ – total volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CED}$ – cohesive energy density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta_{\text{HAN}} = \sqrt{\left(\delta_d^2 + \delta_p^2 + \delta_h^2\right)}$</td>
<td>$\delta_{\text{HAN}}$ – Hansen solubility parameter</td>
<td>79</td>
</tr>
<tr>
<td>$\delta_d$ – partial dispersion component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta_p$ – partial dipole-dipole component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta_h$ – partial hydrogen-bonding component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$(\delta_{d1} - \delta_{d2})^2 + (\delta_{h1} - \delta_{h2})^2 \leq R_o^2$</td>
<td>$R_o$ – radius of interaction sphere in Hansen space</td>
<td>54, 83</td>
</tr>
<tr>
<td>$\delta = \sqrt{\frac{E_{\text{coh}}}{V}} = \sqrt{\frac{(E_{\text{vac}} - E_{\text{bulk}})C}{V}} = \sqrt{\text{CED}}$</td>
<td>$\delta$ – solubility parameter</td>
<td>45</td>
</tr>
<tr>
<td>$E_{\text{coh}}$ – cohesive energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{vac}}$ – energy of molecule in vacuum state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{bulk}}$ – energy of molecule in amorphous state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$ – total volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C$ – unit conversion factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CED}$ – cohesive energy density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_{\text{FH}} = \frac{\Delta H_{\text{mix}}}{kTN\phi_2}$</td>
<td>$x_{\text{FH}}$ – Flory-Huggins interaction parameter</td>
<td>81</td>
</tr>
<tr>
<td>$\Delta H_{\text{mix}}$ – enthalpy change upon creation of a binary mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k$ – Boltzmann constant.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T$ – absolute temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N$ – number of molecules of solvent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\phi_2$ – volume fraction of polymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_{\text{FH}} = \frac{V_{\text{ref}}(\phi_1\text{CED}_1 + \phi_2\text{CED}<em>2 - \text{CED}</em>{12})}{RT}$</td>
<td>$x_{\text{FH}}$ – Flory-Huggins interaction parameter</td>
<td>45</td>
</tr>
<tr>
<td>$V_{\text{ref}}$ – molar volume of the smaller molecule in the binary mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\phi_i$ – volume fraction of component i in the binary mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CED}$ – cohesive energy density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1$ or $2$ – (subscript) indicates compound 1 or 2, respectively</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_{\text{FH}} \approx \frac{VA_{12}}{RT} + \beta$</td>
<td>$x_{\text{FH}}$ – Flory-Huggins interaction parameter</td>
<td>78</td>
</tr>
<tr>
<td>$V$ – the molar volume of the solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$ – gas constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T$ – absolute temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\phi_i$ – volume fraction of component i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_i$ – mole fraction of component i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_{12}$ – enthalpy of mixture of component 1 and 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1$ or $2$ – (subscript) indicates compound 1 or 2, respectively</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta H_{\text{mix}} = x_{\text{FH}}RT\phi_1\phi_2$</td>
<td>$\Delta H_{\text{mix}}$ – enthalpy change upon creation of a binary mixture</td>
<td>81</td>
</tr>
<tr>
<td>$x_{\text{FH}}$ – Flory-Huggins interaction parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$ – gas constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T$ – absolute temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\phi_i$ – volume fraction of component i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_i$ – mole fraction of component i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_i$ – enthalpy of component i at pure-state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_{12}$ – enthalpy of mixture of component 1 and 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1$ or $2$ – (subscript) indicates compound 1 or 2, respectively</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log P = \sum n f_n + \sum \text{correction factors}$</td>
<td>$P$ – partition coefficient</td>
<td>45</td>
</tr>
<tr>
<td>$n$ – functional groups of the molecule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_n$ – hydrophobic fragmental constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a_n$ – incidence of functional group correction factors – a multiple of $n$ and the magic constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{correction factors} = 0.219$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_g = \frac{R}{\sqrt{6}}$, where: $R \approx aN^a$</td>
<td>$R_g$ – radius of gyration of linear polymer</td>
<td>84</td>
</tr>
<tr>
<td>$R$ – end-to-end distance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a$ – bond length of monomer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N$ – degree of polymerization of polymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$ – swelling exponent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Hildebrand SP is related to molecular self-interaction energies and is defined as the square root of the energy of vaporization of a compound per unit volume in the amorphous state (i.e. in the absence of crystallization) at room temperature. According to Hansen, two compounds with similar patterns of polar and hydrogen bonding interactions are predicted to be compatible when the difference in Hildebrand SPs is less than 1.8 cal$^{1/2}$⋅cm$^{-3/2}$ (or 3.7 MPa). However, for compounds with dissimilar patterns of polar and hydrogen bonding interactions, the difference in Hildebrand SPs below which two compounds are predicted to be compatible can be 1.5 or 2-fold larger, especially in solid dispersions. Alternatively, SPs have been used to predict enthalpies of mixing by accounting for the volume fractions of components within a mixture. However, systematic studies comparing the utility of Hildebrand SPs and enthalpies of mixing for rank-ordering materials by miscibility are unavailable. Although the Hildebrand SP is useful for predicting the miscibility of nonpolar materials and materials with sufficiently similar Coulombic interactions, it often performs poorly as an indicator of miscibility for compounds capable of forming hydrogen bonds or salt bridges because these orientation-dependent interactions are not necessarily conserved in different solvents. In these cases, one may apply the Hansen SP, which, although it requires more data and is thus harder to determine than the Hildebrand SP, is useful for predicting the miscibility of polar materials with the potential to engage in hydrogen bonding interactions.

The Hansen SP is based on partial energies of cohesion, dissected into a sum of dispersion, dipolar, and hydrogen bonding components (Equation 1b). Compounds are predicted to be compatible when the difference in Hansen SPs is less than or equal to the
Hansen SP sphere radius (Equation 1c). An example of calculating Hildbrand and Hansen SPs is shown in Table 1.2. Recently, Hansen SPs were used to predict the compatibility of mixtures with more than two components, as described elsewhere. Recently, Hansen SPs were used to predict the compatibility of mixtures with more than two components, as described elsewhere. Further, in analogy to the Hildebrand enthalpy, many groups have calculated Hansen enthalpies of mixing. Here again, systematic studies comparing the utility of Hansen SPs and enthalpies of mixing are unavailable.
Table 1.2: (A) Hildebrand and Hansen solubility parameters \(^88\) of tricaprylin. (B) log\(P\) calculation for tricaprylin with an atom contribution method.\(^90\)

(A) Calculation of Hildebrand and Hansen solubility parameters

\[
\delta_{\text{HIL}} = \sqrt{\frac{\sum E_{\text{coh}}}{V}}
\]

\[
\delta_{\text{HAN}} = \sqrt{\left(\frac{\delta_d^2 + \delta_p^2 + \delta_h^2}{V}\right)}
\]

where:

\[
\delta_d = \frac{\sum F_d^2}{V}, \quad \delta_p = \frac{\sum F_p^2}{V}, \quad \delta_h = \frac{\sum F_h^2}{V}
\]

<table>
<thead>
<tr>
<th>Fragments</th>
<th>Frequency</th>
<th>(V) (cm(^3)mol(^{-1}))</th>
<th>(F_d) (J(^{1/2})cm(^{3/2})mol(^{-1}))</th>
<th>(F_p) (J(^{1/2})cm(^{3/2})mol(^{-1}))</th>
<th>(E_h) (Jmol(^{-1}))</th>
<th>(E_{\text{coh}}) (Jmol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(_3)</td>
<td>3</td>
<td>100.5</td>
<td>1260</td>
<td>0</td>
<td>0</td>
<td>14130</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>20</td>
<td>322</td>
<td>5400</td>
<td>0</td>
<td>0</td>
<td>98800</td>
</tr>
<tr>
<td>CH</td>
<td>1</td>
<td>1.0</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>3430</td>
</tr>
<tr>
<td>COO</td>
<td>3</td>
<td>54.0</td>
<td>1170</td>
<td>720300</td>
<td>21000</td>
<td>54000</td>
</tr>
</tbody>
</table>

\(\delta_d = 16.6351, \quad \delta_p = 1.78487, \quad \delta_h = 6.646, \quad \delta_{\text{HAN}} = 18.002 \) (J\(^{1/2}\)cm\(^{3/2}\)mol\(^{-1}\)), \quad \delta_{\text{HIL}} = 18.928 \) (J\(^{1/2}\)cm\(^{3/2}\)mol\(^{-1}\)).

(B) Calculation of log\(P\) using atom contribution method

\[
\log P = \sum_{i=1}^{n} n_i a_i
\]

where:

- \(n_i\) - number of atoms of type \(i\)
- \(a_i\) - contribution of an atom of type \(i\).

<table>
<thead>
<tr>
<th>Type</th>
<th>Description (^a)</th>
<th>Frequency</th>
<th>Hydrophobic contribution (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C in CH(_3)-R</td>
<td>3</td>
<td>-1.8111</td>
</tr>
<tr>
<td>2</td>
<td>C in CH(_2)-R(_2)</td>
<td>18</td>
<td>-7.731</td>
</tr>
<tr>
<td>40</td>
<td>C in COO (R-C(=X)-X</td>
<td>3</td>
<td>0.0834</td>
</tr>
<tr>
<td>6</td>
<td>C in R-CH2-X</td>
<td>2</td>
<td>-1.6376</td>
</tr>
<tr>
<td>8</td>
<td>C in R2-CH-X</td>
<td>1</td>
<td>-0.5995</td>
</tr>
<tr>
<td>46</td>
<td>H attached to (^c)C(_{sp^3}), having no X attached to next C</td>
<td>39</td>
<td>16.5126</td>
</tr>
<tr>
<td>47</td>
<td>H attached to (^c)C(_{sp^3})</td>
<td>5</td>
<td>1.805</td>
</tr>
<tr>
<td>51</td>
<td>H attached to (^c)(\alpha)-C (^d)</td>
<td>6</td>
<td>1.1214</td>
</tr>
<tr>
<td>58</td>
<td>O in =O</td>
<td>3</td>
<td>-0.7419</td>
</tr>
<tr>
<td>60</td>
<td>O in Al-O-Ar, Ar(_2)O, R…O…R, R-O-C=X</td>
<td>3</td>
<td>0.5814</td>
</tr>
</tbody>
</table>

\(\log P = 7.5827\)

- \(\alpha\)-C may be defined as a C attached through a single bond with -C=X.

---

\(\text{H2C}\)\(\text{CH}\)\(\text{H2C}\)\(\text{O C}\)\(\text{O (CH2)}_6\)\(\text{CH3}\)

\(\text{H2C}\)\(\text{O C}\)\(\text{O (CH2)}_6\)\(\text{CH3}\)

---

\(\text{H2C}\)\(\text{CH}\)\(\text{H2C}\)\(\text{O C}\)\(\text{O (CH2)}_6\)\(\text{CH3}\)

---

\(\text{H2C}\)\(\text{CH}\)\(\text{H2C}\)\(\text{O C}\)\(\text{O (CH2)}_6\)\(\text{CH3}\)

---

\(\text{H2C}\)\(\text{CH}\)\(\text{H2C}\)\(\text{O C}\)\(\text{O (CH2)}_6\)\(\text{CH3}\)
Hansen SPs and Hansen SP-based enthalpies of mixing have been used to estimate the compatibility of materials for polymer-lipid hybrid nanoparticles. Specifically, enthalpies were predicted for mixing a drug-polymer complex (verapamil HCl in dextran-sulfate-sodium) with fifteen different lipids including fatty acids, triglycerides, glycerol esters and mixtures of glycerol esters.\textsuperscript{85} Based on these calculations, dodecanoic acid and monoglyceryl behenate were identified as the most suitable components to solubilize the drug-polymer complex.\textsuperscript{85} Nine lipids were selected for experimental evaluation and, of these, the apparent partition coefficient of the drug-polymer complex between lipid-like substrates and the aqueous phase was greatest for dodecanoic acid. However, the drug-polymer complex had poor relative affinity for Compritol®ATO 888, which is a mixture of glycerol esters with 15 % of monoglyceryl behenate. Of the materials evaluated experimentally, the top three predictions were reproduced in rank order, although several of the materials predicted to be more suitable (e.g. pure monoglyceryl behenate) were not evaluated.\textsuperscript{85}

Both the Hildebrand and Hansen SPs can be obtained relatively quickly by using group contribution methods.\textsuperscript{88} They are therefore well-suited to rapidly eliminate materials that are likely to be incompatible, thereby enriching the library of materials under consideration in the early stages of formulation development prior to application of more computationally and/or experimentally expensive methods.\textsuperscript{49, 57, 87, 91} SPs are, however, based on the assumptions of regular solution theory for liquids and do not take into account the effects of entropy and the free volume of amorphous solids.\textsuperscript{59} Further, SPs do not account for any dependencies on concentration, conformation, or unique interactions between molecules that may be present in binary mixtures. SPs are, therefore,
sometimes inaccurate. Indeed, studies have shown that SPs calculated based on group contribution methods only provide accurate predictions when comparing materials that have similar chemical structures.\textsuperscript{48} Similarly, Lipinski postulated that prediction of the aqueous solubility of drug-like compounds is more successful with neutral compounds and within a series of compounds with similar chemical structures.\textsuperscript{1}

1.3.2. Flory-Huggins interaction parameter

The Flory–Huggins (FH) theory\textsuperscript{44, 45, 92} was established with the intention to describe the thermodynamic behavior of non-idealized polymer-solvent solutions. The FH interaction parameter, $\chi_{FH}$, is a dimensionless quantity which represents the interactions that contribute to the enthalpy of mixing of a polymer and a solvent. Compounds are predicted to be miscible when $\chi_{FH}$ is less than 0.5 (or phase separated when $\chi_{FH}$ greater than 0.5).\textsuperscript{45} Experimentally, $\chi_{FH}$ can be determined from the enthalpy change associated with creation of a binary mixture as shown in Equation 2a,\textsuperscript{45} although this equation is only useful for validation since there are more accurate experimental methods to determine miscibility. For predictions, $\chi_{FH}$ can be obtained from MC\textsuperscript{81} and MD\textsuperscript{82} simulation, as outlined in Equation 2b. Finally, the $\chi_{FH}$ value can be calculated using group contribution implementations of Hildebrand and Hansen SPs, as outlined in Equation 2c,\textsuperscript{45, 54, 78} although the inaccuracies of these concentration (and possibly conformation and hetero-interaction) independent SPs are, in this case, propagated to $\chi_{FH}$.\textsuperscript{52} These inaccuracies are important because many studies have shown that the $\chi_{FH}$ varies as a function of the volume fraction of the solute and solvent components in the
mixtures. Importantly, the FH theory has recently been extended to predict the miscibility of drugs and carrier materials.

Recently, Dwan’Isa et al. calculated $\chi_{FH}$ values from Hansen SPs for drug-polymer pairs. This was a systematic study of nineteen drugs and segments of two polymers: hydrophilic monomethoxy PEG (MePEG), and a hydrophobic copolymer of equimolar randomly-distributed poly(ɛ-caprolactone) and trimethylene carbonate (PCL-co-TMC). The linear diblock copolymer MePEG-b-(PCL-co-TMC) can form micelles with an inner hydrophobic PCL-co-TMC core and an outer hydrophilic MePEG shell. To predict drug loading in these micelles, $\chi_{FH}$ was separately evaluated for the drug with the hydrophobic PCL-co-TMC, $\chi_{FH_{hydrophobic}}$, and with the hydrophilic MePEG, $\chi_{FH_{hydrophilic}}$. The root-sum-squared (RSS) $\chi_{FH}$ was then used to predict the rank order drug loading in the micelles, where $RSS = \left( \chi_{FH_{hydrophobic}}^2 + \chi_{FH_{hydrophilic}}^2 \right)^{0.5}$. Drug loading was evaluated experimentally for eight hydrophobic drugs with low water solubilities, ranging from 0.01 mg/mL to 1 mg/mL, in MePEG-b-(PCL-co-TMC) micelles and compared to the theoretical rank order of loading for these drugs in the micelles. Rank order agreement between theoretical predictions and experimental results was obtained for the top three predictions of drug loading capacity (3.7, 10.5 and 19.6 mg/mL for indomethacin, cimetidine, and ketoprofen, respectively). These three drugs were not the most hydrophobic drugs, indicating that the $\chi_{FH}$ predictions were useful. Incorrect rank-ordering of the other five drugs can be attributed to several factors including the ad-hoc combined theoretical score, conditions of the experimental study, and limitations of the theory related to both drug rigidity and the interfacial tension between molecules.
Nevertheless, this method appears to be a good initial step for library design and material acquisition.

In another study, Mahmud et al. calculated $\chi_{FH}$ values from Hansen SPs for the anti-cancer drug cucurbitacin I and various hydrophobic core-forming polymers including PCL, poly(α-benzylcarboxylate-ε-caprolactone) (PBCL) and poly(α-cholesteryl carboxylate-ε-caprolactone) (PChCL). Based on the calculated $\chi_{FH}$ values, PChCL and PCL were identified as the most and least suitable core-forming polymers, respectively. This finding agreed well with the experimental molar loading ratios of drug to core-forming repeat unit in the micelles, which were 3 %, 8 %, and 15 % in MePEO-b-PCL, MePEO-b-PBCL, and MePEO-b-PChCL micelles, respectively. Nevertheless, MePEO-b-PBCL exhibited the best controlled release of cucurbitacin I. Mahmud et al. interpreted this in light of the fact that more negative $\chi_{FH}$ values represent stronger interactions and better compatibility between the drug and material. Nevertheless, the release rate is a kinetic quantity and there is no a priori reason to assume that the release rate is related to $\chi_{FH}$. Further, predicting the drug release rate is complicated by the many contributing factors including the state of micelle core, the state of the drug in the micelle core, and the hydration of the core or core/corona interface. Importantly, rates of drug uptake and release can be rigorously derived from molecular simulations.

While the FH model is useful, it is founded on some assumptions that may lead to discrepancies between experimental data and theoretical predictions of drug-material interaction based on $\chi_{FH}$. Specifically, FH theory assumes that there is a random distribution of polymer segments and that the attraction between polymer and solvent is negligible and thus the volume of mixing will be unchanged upon mixing of polymer and
There is thus a need to improve and extend FH theory in order to more accurately predict drug-material interactions.

1.3.3. Lipophilicity

Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. Lipophilicity can be determined by measuring the partition coefficient $P$ which is the ratio of solute concentrations in binary phases of organic and aqueous solvents, such as octanol and water, under equilibrium conditions. Because the value $P$ ranges widely, the lipophilicity of compounds is represented as the logarithm of $P$, $\log P$. In addition to the aforementioned experimental evaluation, $\log P$ can be predicted using a theoretical group contribution approach first introduced by Rekker and Mannhold, which they termed the fragmental method (Equation 3). Detailed methods for theoretical calculation of $\log P$ can be found in review articles by Leo and Lipinski et al. Successful applications of Rekker and Mannhold’s equation for the theoretical calculation of $\log P$ have been reported for various drugs and small molecules. Alternatively, atom contribution can be used to predict $\log P$ by summing the single-atom contributions. An example of calculation of $\log P$ via an atom contribution method is demonstrated in Table 1.2.

Hydrophobicity drives the association of non-polar groups or molecules in an aqueous environment. The tendency of water to exclude non-polar molecules arises because non-polar groups cannot take part in hydrogen bonding with water. While most hydrophobic compounds are also lipophilic, there are compounds such as fluorocarbons...
that are both hydrophobic, for the aforementioned reasons, and lipophobic, because they cannot take part in the strong dispersion forces among hydrocarbons.\textsuperscript{99} The weak dispersion force of fluorocarbons is contributed from the high electronegativity of fluorine which reduces the polarizability of the atom.\textsuperscript{99} Nevertheless, major components of hydrophobicity and lipophilicity remain intertwined and the terms are often used interchangeably. Lipophilicity and hydrophobicity can be used to predict drug retention in a formulation\textsuperscript{49,100} or drug permeability through a membrane.\textsuperscript{38}

Theoretical calculation of $\log{P}$ is a valuable model for rapid prediction of the lipophilicity of the compounds. The group contribution approaches, however, may produce inaccurate $\log{P}$ values because they do not consider the conformation of the compound.\textsuperscript{97} Further, the polarity of a compound is not additive.\textsuperscript{97} Nevertheless, theoretical calculation of $\log{P}$ may be used for rapid screening prior to time consuming and expensive experimental studies.

\textbf{1.3.4. Architectural and conformational contributions to drug-material compatibility}

The efficient and stable encapsulation of hydrophobic compounds into nanoparticles is governed not only by the solubilities of drugs and materials,\textsuperscript{49,63,72} but also by other physical properties. These properties include rigidity, conformation, the MW of the drug and the materials.\textsuperscript{52,61} As an alternative to analytical methods, molecular simulations provide ensemble representations of biomaterials from which one can extract properties such as size, conformation,\textsuperscript{40,65} and the interfacial structure of drug-material or material-material pairs.\textsuperscript{73,101} Molecular simulation can reveal the fundamental
interactions governing drug-polymer assembly, elucidating the physical and chemical features that can be modified to influence drug loading or release efficiency.\textsuperscript{63, 64, 69} The ensemble of configurations of drugs interacting with the core-forming materials is especially useful to understand the efficiency by which nanoparticles encapsulate a specific drug.\textsuperscript{63, 64}

Interestingly, Hildebrand SPs can be evaluated from simulation (Equation 1c).\textsuperscript{48, 52, 53} In this case, conformation and intra- and inter-molecular interactions, but not concentration, are treated explicitly. To our knowledge, the Hansen SP has not been calculated from molecular simulations.

A unique aspect of molecular simulation is that it can explicitly evaluate the conformations of the components, both individually and as a binary mixture. It is, then, not surprising that SPs based on simulations have been more accurate than those obtained analytically.\textsuperscript{48} Interestingly, the disparity between the SP values obtained from simulation and semi-empirical methods increases with MW, indicating that the dependence of solubility on conformation and molecular orientation increases with size.\textsuperscript{48}

Recently, Pajula et al. used MC simulations to predict the $\chi_{FH}$ values for binary mixtures of 34 small drug molecules.\textsuperscript{102} Temperature-dependent $\chi_{FH}$ values were generated based on free energies calculated from many configurations of interacting drug pairs. Drug pairs were predicted to be miscible and immiscible for negative and positive $\chi_{FH}$ values, respectively. Predictions were validated for 27 drug-excipient pairs by hot-stage polarized light microscopy, assuming that a binary mixture is thermodynamically miscible when it does not crystallize following heat-cool treatment.\textsuperscript{102} The immiscible predictions were correct for all 16 selected pairs where 13 of these pairs are strongly
predicted to be immiscible ($4.5 < \chi_{FH} < 26.6$) and 3 pairs are weakly predicted to be immiscible ($\chi_{FH}$ of 0.1 and 0.6). The miscible predictions were correct in only 8 of 11 selected pairs where the 8 experimentally miscible drug pairs have a predicted $-6.5 < \chi_{FH} < -0.4$ and the 3 experimentally immiscible drug pairs incorrectly predicted as miscible had $\chi_{FH}$ values of -0.2, -0.4 and -2.2. Therefore, while this method is imperfect, it remains an excellent method to accelerate material development.

Generally, a realistic representation of drug delivery systems based on polymer micelles comprises millions of atoms including explicit solvent. Atomistic simulations may not be feasible due to lack of computer resources. To overcome computational limitations, molecular simulations have been applied to single-chain block copolymers or low MW oligomeric unimers, for the prediction of drug-polymer compatibility. To this end, Patel et al. reported an in silico method for predicting the compatibility of poorly water soluble drugs and poly(ethylene oxide)-b-PCL (PEO-b-PCL) by means of the $\chi_{FH}$ parameters of drug-polymer pairs.\textsuperscript{52, 53} Patel et al. calculated concentration-independent and concentration-dependent $\chi_{FH}$ values from both SPs and Hildebrand enthalpies of mixing derived from MD simulations using Equation 2c and 2d, respectively.\textsuperscript{52, 53} To circumvent limitations on the achievable simulation timescales, the authors reduced computational expense by using, in place of PEO-b-PCL micelles, a simulation system containing a drug and a single unimer of PEO-b-PCL that was allowed to interact with itself over periodic boundary conditions. These studies revealed the interactions of binary mixtures of a unimeric diblock copolymer chain of PEO-b-PCL with various concentrations of hydrophobic drugs including fenofibrate, nimodipine, and cucurbitacin B and I.\textsuperscript{52, 53} At low drug concentration ($\leq 40$ % w/w drug/polymer), the simulation
results agreed well with the experimental solubilities of fenofibrate and cucurbitacin in diblock copolymer PEO-b-PCL micelles, and nimodipine in triblock copolymer PCL-b-PEO-b-PCL micelles. SPs predicted by analytical group contribution methods, however, did not provide accurate indications of experimental solubilities.

Using a similar simulation-based method, Patel et al. also predicted that hydrophobic drugs that contain only hydrogen acceptor groups, such as fenofibrate and nimodipine, engage better in hydrogen bonding with linear PEO-b-PCL than with the branched copolymer PEO-b-3PCL. In contrast, cucurbitacins were predicted to be more compatible with branched PEO-b-3PCL than with linear PEO-b-PCL. Based on these findings, Patel et al. proposed that the increase in compatibility between cucurbitacins and PEO-b-3PCL was due to the increase in hydrogen bonding between the drug and branched PEO-b-3PCL, although the experimental solubilities of the investigated drugs in the PEO-b-3PCL are not available for validation. Generally, the influence of the MW of PEO and PCL on the compatibility of drugs and PEO-b-PCL depends on the tendency of a drug to associate with PEO and/or PCL blocks. Specifically, the compatibility between drugs and polymers was enhanced when the MW of PCL blocks increased from 1.25 kDa to 2.5 kDa. Also, increasing the MW of PEO blocks from 1.25 kDa to 2.5 kDa decreased the χFH-predicted compatibility of PEO-b-PCL and fenofibrate or nimodipine and increased the χFH-predicted compatibility between PEO-b-PCL and cucurbitacin B. The enhanced compatibility of cucurbitacins, hydrophobic drugs with multiple hydrogen acceptor and donor groups, was attributed to an increase in the number of hydrogen bonds and polar interactions between the drugs and PCL when the ratio of PCL/PEG was increased from 0.5 to 2.
Overall, these studies suggest that the compatibility between drugs and core-forming materials can be enhanced by modification of the MW, the architecture, or the hydrogen-bonding capabilities of the delivery material. The discrepancy between experimental data and predictions from the aforementioned molecular simulation studies are likely due, in part, to the absence of water. Further complicating the resolution of such discrepancies, evaluations of convergence are absent from many simulation studies. The primary drawback to molecular simulation is the large computational cost associated with atomistic simulations in explicit solvent. It is thus not possible to evaluate thousands of drug-material pairs by molecular simulation. However, the method remains an excellent tool for the evaluation of candidate pairs that have been pre-screened by analytical models or the discerning eye of a pharmaceutical scientist.

1.3.5. Covalent linkage of formulation-like moieties to drugs

Drug loading and retention can be significantly improved by increasing the compatibility between the drug and the delivery material. The retention of hydrophobic compounds in the core of nanoparticles is in part governed by the partition coefficient of the hydrophobic molecule between the core and the aqueous environment. There are many ways to improve drug-material compatibility, but one of the simplest and potentially least costly is to chemically conjugate a formulation-like moiety to the drug, thus generating a prodrug with enhanced delivery material compatibility. To increase efficiency, computational methods have been used to direct drug modification.

Forrest et al. used SPs to predict the loading of lipophilic prodrugs of geldanamycin in polymeric micelles composed of PEG-\(b\)-PCL. Geldanamycin is an
anticancer agent that, unmodified, is insoluble in the core of PEG-b-PCL micelles.\textsuperscript{72} Geldanamycin and PCL have Hansen SPs of 23.1 and 20.2, respectively. A $\chi_{FH}$ value of 1.33, calculated from Hansen SPs, was obtained for the geldanamycin-PCL pair by using Equation 2c. The conjugation of a fatty acid to geldanamycin resulted in geldanamycin prodrugs with Hansen SPs between 20.4 and 21.6, and $\chi_{FH}$ values for the prodrug-PCL pairs that improved to 0.02 and 0.24. The accuracy of these theoretical predictions varied depending on the hydrocarbon chain length of the conjugated fatty acids. Specifically, unexpectedly low drug loading was obtained when encapsulating C6 acyl conjugates in PEG-b-PCL micelles. Nevertheless, successful encapsulation of C12 and C16 acyl conjugates in PEG-b-PCL micelles were reported with a loading capacity up to 50-fold higher than that obtained with unmodified geldanamycin.\textsuperscript{72}

Applying similar ideas to a nanoemulsion formulation, a combination of analytically predicted SP and log$P$ values was used to rationally design a prodrug of the hydrophobic anticancer agent docetaxel.\textsuperscript{49} Theoretical results suggested that conjugation of fatty acids with chain lengths similar to the core-forming materials of a triglyceride-based oil emulsion would increase the lipophilicity of the drug, thus decreasing the difference in their SP values and enhancing the compatibility of the drug-material pair. Experimentally, conjugation of dodecyl fatty acid(s) to docetaxel was found to increase the solubility of the drug in triglyceride by 8-fold and its loading efficiency in the nanoemulsion by 10-fold.\textsuperscript{49} Further, theoretical and experimental log$P$ values indicated that conjugates with multiple conjugated fatty acids were even more lipophilic. Nevertheless, only the monosubstituted prodrug was hydrolyzed under biologically relevant conditions to yield an activity similar to that of the parent drug.\textsuperscript{49}
Group contribution methods are excellent approaches for computing SPs and predicting the logP of drugs and drug derivatives. These parameters provide relatively accurate predictions for compounds with similar chemical structures.

1.3.6. Predicting drug loading and retention

Controlling drug release is one of the key requirements of a successful drug formulation. Premature drug release from the formulation may lead to systemic side effects, due to the distribution of drug to non-target tissues, as well as inefficient drug accumulation at the target site.\textsuperscript{11, 12, 106}

Recently, Costache \textit{et al.} used docking techniques to determine an ensemble of poses of drugs interacting with core-forming polymers of nanospheres and predicted the interaction energy of hydrophobic drugs and tyrosine-derived oligomers.\textsuperscript{64} Prior to docking calculations, the aggregation state of the hydrophobic oligomer was equilibrated by performing MD simulations on hydrated single and multiple chains of ABA triblock copolymers followed by the removal of the PEG blocks.\textsuperscript{64} Here, A = PEG and B = oligo(desaminotyrosyl-tyrosine octyl ester suberate). In this study, Costache \textit{et al.} reported a correlation between the experimental maximum drug loading in the triblock copolymer nanospheres and the theoretically calculated polymer-drug binding energies, where the drug models were curcumin, paclitaxel, and vitamin D3. Consistent with other theoretical studies,\textsuperscript{63, 71} the binding affinities of hydrophobic drugs to the core-forming polymers were proportional to the number of hydrogen bonds, the number of aromatic interactions of the drug-polymer pairs, and the hydrophobicity of the drug. Other features such as size and flexibility also influence binding affinities and location of the drug.
within the delivery system. In particular, highly hydrophobic (predicted log$P = 7.9$) and flexible vitamin D3 was predicted to penetrate into the hydrophobic core of the nanosphere delivery system and have the most favourable docking energy, $D (D = −10.3 \text{kcal/mol})$, erroneously referred to as a binding free energy by Costache et al.64 This finding agreed well with experiment, where vitamin D3 has the highest drug loading in the nanosphere. The less flexible and less hydrophobic curcumin (predicted log$P = 3.6$) was similarly found to penetrate into the hydrophobic core of the nanosphere. The predicted docking energy of curcumin ($D = −7.2 \text{kcal/mol}$) to the nanosphere was less favourable than vitamin D3 and experimentally, curcumin loaded less into the nanosphere than did vitamin D3. Finally, the more bulky and rigid paclitaxel (predicted log$P = 3.2$) was predicted to preferably interact with the surface of the hydrophobic core of the nanospheres and have an even less favourable docking energy ($D = −4.4 \text{kcal/mol}$). In agreement with molecular simulation, paclitaxel was found to have the lowest experimental drug loading in the nanospheres.64

Current research suggests that hydrogen bonding enhances drug loading and slows drug release,64,71 although rank ordering can be complicated by the influence of other factors, such as lipophilicity.49 In this context, Sutton et al. demonstrated that a single doxorubicin forms 5 to 6 hydrogen bonds with a hydrophobic aggregate of PCL and 3 to 4 hydrogen bonds with a hydrophobic aggregate of PLA, although water molecules were not present in this simulation.63 The authors proposed that the increased number of hydrogen bonds formed by doxorubicin in PEG-$b$-PCL micelles is responsible for the slower drug release in this system as compared to PEG-$b$-PLA micelles.63
Similarly, the MD simulation of docetaxel molecules solvated by pharmaceutical excipients revealed that the solubility of docetaxel in small molecule excipients, such as triglycerides and vitamin E, increases with an increasing number of hydrogen bonds between the drug and the excipients as shown in Figure 1.1. Nevertheless, better experimental drug retention was observed with a highly hydrophobic excipient that formed fewer hydrogen bonds with docetaxel. Similar observations were also reported based on a combination of MD simulation and molecular docking. Indeed, the lipophilicity of materials has, in some cases, contributed to drug loading and retention to a greater extent than hydrogen bonding capability, and the number of hydrogen bonds between the drug and excipient does not always correlate with the rate of drug release, especially at high drug loading where drug aggregation may occur within the formulation.

Properly weighting the many competing factors that influence drug-material compatibility is one of the great challenges to theoretical methods.
Figure 1.1: Hydrogen bonding between docetaxel (DTX) and small molecule excipients from MD simulations of a DTX-excipient binary mixture. Excipients that do not form hydrogen bonds with DTX are not shown. The excipient is represented in green and docetaxel with multicoloured lines (grey carbon, white hydrogen, red oxygen, and blue nitrogen). The intra- and intermolecular hydrogen bonds are indicated by thin white and thick yellow arrows, respectively. Functional groups involved in hydrogen bonds are highlighted by licorice representation. Explicit solvent is omitted for clarity.
1.4. Optimizing Materials

The overwhelming importance of polymer behavior in nanotechnologies has motivated the construction of many theoretical models to predict the conformation and size of polymers in various media. There is thus a wealth of theoretical studies that provide insight into the aggregated and self-assembled structures of formulation materials.

Section 1.4.1 reviews the use of analytical and simulation models to predict the properties of biomaterials with simple structures. The remaining sections in this review describe the application of molecular simulation to study the atomistic structure of multi-component nano-emulsions (section 1.4.2), block copolymer micelles (section 1.4.3), high MW molecules with complicated structures such as star copolymers (section 1.4.4) and dendrimers (section 1.4.5).

1.4.1. Phenomenological models and simulations of linear polymers

In the 1950’s, Flory put forth the random flight model for free linear polymers, in which polymer size depends on both polymer length and solvent suitability. This model was derived from Debye theory and predicts the radius of gyration, \( R_g = R/\sqrt{6} \), where \( R \) is the root mean squared end-to-end distance of the polymer. Here, \( R \) scales with the effective bond length of the monomer, \( a \), and the degree of polymerization of the polymer, \( N \), according to a power law such that \( R \approx aN^\alpha \), where \( \alpha \) is the swelling exponent. In a good solvent, \( R \) is known as the Flory radius, \( R_F \), and \( \alpha \) is equal to 0.588. In a mediocre solvent (theta solvent), the polymer behaves ideally and exists as a Gaussian coil with an \( \alpha \) value of 0.5. In a poor solvent, the polymer collapses and has an
$\alpha$ value of $\frac{1}{3}$. While such simplifications are useful, they are also imperfect. When $\alpha$ values are back-calculated from experimental measures of size, the $\alpha$ values of polymers in good solvents depend somewhat on the polymer composition and MW. For example, gel permeation chromatography and size exclusion techniques provide an $\alpha$ value of 0.571 for aqueous solutions of linear PEO with a MW of 25 KDa to 120 KDa. From the same study, $\alpha$ values of 0.523 were obtained for linear PEG with a MW of 0.2 KDa to 7.5 KDa. Similarly, Lee et al. reported simulations of a series of hydrated PEO polymers with various degrees of polymerization using MD and a coarse grained (CG) hydrodynamic bead model. In this study, the conformation of PEO was described as an ideal chain with $\alpha = 0.515$ for $0.44 \text{kDa} < \text{MW}_{\text{PEO}} < 1.63 \text{kDa}$. Here, MD simulation outperformed the hydrodynamic bead model as a tool for predicting the size of PEO molecules, yielding hydrodynamic radii and diffusion constants of PEO in better agreement with experimental results. In a separate CG simulation study, Lee et al. reported an $\alpha$ value of 0.57 for $1.63 \text{kDa} < \text{MW}_{\text{PEO}} < 7.0 \text{kDa}$.

Whereas Flory’s random flight model is limited to polymers free in solution, the conformation of polymers attached to a planar surface can be predicted by de Gennes theory. According to this theory, grafted polymers adopt so-called mushroom and brush regimes in a good solvent at low and high grafting density, respectively. In the mushroom regime, named for the predicted mushroom-like shape of each polymer, the grafted polymers are separated by a distance $D > R_F$ and occupy a “half-sphere with a radius comparable to $R_F$ of a Gaussian coil.” In the brush regime, named for the predicted bristle-like appearance of the polymers extending outward from the surface, $D$
< \( R_F \) and the grafted polymers adopt a more extended configuration as they horizontally occlude one another.\(^{112} \)

Torchilin and Papisov hypothesized that each PEG molecule attached to a surface of liposome occupies a “dense cloud” of conformations over the liposome surface and protects the liposome from fast clearance from the bloodstream by the host mononuclear phagocytic system.\(^{11, 12, 15} \) Based on de Gennes theory,\(^{111-113} \) Torchilin and Papisov proposed that the protected surface area, \( A \), provided by a single PEG block grafted to a PEGylated liposome in aqueous solution can be estimated by

\[
A = \pi \times \frac{R_F^2}{4}
\]

assuming that PEG blocks adopt random coil conformations.\(^{25} \) Therefore, for a given surface area of liposome, it is possible to estimate the PEG:lipid molar ratio required to achieve full protection of the liposome surface. However, studies have shown that this model is only approximate for PEG grafted spherical surfaces.\(^{40, 65} \)

Daoud and Cotton theory extends de Gennes theory to star shaped polymer models.\(^{119} \) This theory predicts that the radius of a star shaped polymer is smaller than that of a linear polymer of the same MW.\(^{119} \) Significantly, this prediction was confirmed by experimental and theoretical studies of free linear PEG blocks and amphiphilic PEG-\( b \)-PCL star shape copolymers in water.\(^{40} \)

In spite of the fact that the theories of de Gennes, Torchilin and Papisov, and Daoud and Cotton are most often applied to curved surfaces, these theoretical models exclude the influence of the radius of the sphere – an inauspicious assumption given that deviation from planarity significantly influences the conformation of the outer layers of a core-shell nanoparticle.\(^{114} \) Surface curvature may influence the adsorption of hydrophilic polymers on hydrophobic surfaces.\(^{40} \) Indeed, the conformations of polymers grafted to a
curved surface are theoretically predicted to depend not only on the chain length of the polymer, but also on the radius of curvature of the surface.\textsuperscript{114} Specifically, when the ratio of the radius of curvature to the thickness of the grafted polymer layer is much less than one, the $R_g$ of grafted polymers grow as a function of the degree of polymerization of the polymer with $\alpha$ equal to 3/5.\textsuperscript{114} There is a clear need for extensions of available theoretical methods to explicitly account for surface curvature.

\subsection*{1.4.2. Nano-emulsions}

In order for a nano-emulsion to consist of stable particles of a desired size, the interactions between the core- and shell-forming materials must be optimized.\textsuperscript{120} This can be done experimentally by selecting compatible components and determining the appropriate mixing ratio,\textsuperscript{106, 109} although this approach is very time consuming. Current theoretical efforts are directed at understanding how nano-emulsions integrate components at the atomistic level, collecting information that will be useful for future rational design.

To this end, oil-in-water nano-emulsions were recently simulated by Lee \textit{et al.}\textsuperscript{73} and, separately, by Henneré \textit{et al.}\textsuperscript{101} In these studies, the nano-emulsion systems were represented by a planar model which is layered as water, phospholipid, oil, phospholipid, water.\textsuperscript{73} The oil phase was composed of trilinoleylglycerol,\textsuperscript{101} or perfluorooctylbromide (PFOB),\textsuperscript{73} and the phospholipid was palmitoyloleoylphosphatidylcholine (POPC).\textsuperscript{73, 101} In these simulations, the head group of POPC was fully hydrated, and the hydrocarbon tails of POPC interacted favourably with the hydrophobic triglyceride tails, as expected.\textsuperscript{101, 121, 122} In contrast to triglycerides, perfluorocarbons are lipophobic \textsuperscript{99} and are
expected to be immiscible with POPC. However, an experimental study by Yokoyama et al. showed that the miscibility of perfluorocarbons with hydrocarbons increases when the perfluorocarbons are significantly shorter than the hydrocarbons. Interestingly, Lee’s aforementioned study of the PFOB/POPC nano-emulsion system indicated that the FC8 perfluorocarbon tail of PFOB interacts with the C16 and C18 hydrocarbon tails of POPC. This result suggested that this simulation captured important interactions as they exist in vitro. This PFOB/POPC nano-emulsion model was then used to investigate the quenching mechanism of melittin tryptophan. In agreement with experiment, the tryptophan side chain of melittin was located within the POPC layer. Further, based on the radial distribution of the bromine atoms of PFOB around tryptophan residues, the bromine was very close to direct contact with the tryptophan. These results are consistent with the known quenching mechanism of tryptophan which is due to the collision of the tryptophan with bromine. Overall, this study demonstrated that atomistic simulations can reproduce the interactions and the quenching mechanism of tryptophan within the PFOB/POPC nano-emulsion system at the molecular level.

Applied methods yield structures, but no thermodynamic information. To further quantitatively determine the importance of constituent interactions, efficient generalized-ensemble simulation algorithms can be used to construct the free energy profiles that govern these interactions.

1.4.3. Linear block copolymer micelles

Linear, amphiphilic diblock and triblock copolymers have emerged as the materials of choice for use in a wide range of biomedical applications, including
Polymeric micelles comprise a hydrophobic core, which can load and store drugs as cargo, and a hydrophilic shell, which surrounds the hydrophobic core and hinders particle uptake by the host mononuclear phagocytic system. Molecular simulations have been employed to investigate the structure, dynamics, and self-aggregation properties of polymeric micelles, with or without drugs.

Kuramochi et al. used all-atom MD simulations to study the structure of a spherical micelle composed of 20 chains of the linear diblock copolymer PEG$_1_{11}$-$b$-poly($\gamma$-benzyl L-glutamate)$_9$ (PEG$_{11}$-$b$-PBLG$_9$) in explicit water. In addition, Huang et al. investigated glycyrrhetinic acid modified PEG-$b$-PBLG micelles as drug carriers for doxorubicin. According to Kuramochi et al., a slightly elliptical micelle structure is formed after 7 ns of simulation, with a hydrophobic PBLG inner core and a hydrophilic outer PEG shell. The core-forming polymer, PBLG, is hydrophobic due to its benzyl group side-chains. Nevertheless, it has a backbone made of hydrophilic esters and amide groups, and is capable of forming hydrogen bonds with water. In agreement with NMR and other MD studies of free linear PEG, the PEG blocks of the micelle presented in this study were highly hydrated and adopted a helical conformation. Further, this study revealed that the benzyl groups, which contribute significantly to hydrophobic interactions, were preferentially located near the center of the hydrophobic core. Based on the radial density distribution, some water molecules dynamically penetrated into the hydrophobic PBLG core and formed a hydrogen-bonded network with the ester and amide groups in the backbone of the hydrophobic core (Figure 3a). The transient presence of water molecules within the hydrophobic core is unlikely to have been
predicted by an analytical group contribution method. Structurally, the PBLG chain adopted an α-helix conformation that was stabilized by six to eight hydrogen bonds within a PBLG block. Overall, the micelle was stabilized by multiple hydrogen bonds between water and the PEG blocks (Figure 3b) and hydrophobic interactions within the PBLG core.68

In another study, Mathias et al. used a combination of NMR and MD simulation to investigate the location of an electron-spin labelled hydrophobic drug, chlorambucil-tempol adduct, in fluoroalkyl-linker and fluoroalkyl-linker-PEG micelles 70 in which the fluoroalkyl hydrophobic segment, CF₃(CF₂)₅CH₂CH₂-, is expected to form an inner core that excludes water. In order to reduce computational expense during simulations, explicit water was omitted in favour of a restraining force on the CF₃ groups to assist the formation of fluoroalkyl-linker or fluoroalkyl-linker-PEG micelles. Under this restraining force, the hydrophobic fluorocarbons cluster together and form the inner core of the micelle, whereas the relatively less hydrophobic linker, isophorone diurethane, forms an interface between the inner core and the hydrophilic PEG shell.70 In these simulations, the system was simulated with a single drug initially placed successively at the outside of micelle, in the inner fluoroalkyl core, or at the core-PEG interface, each successively with and without distance restraints based on NMR data. During unrestrained simulation, the drug was shown to migrate to the isophorone diurethane interface region of the micelle.70 Overall, this study provides a simplified model to capture the preferred interactions and localization of a drug among a variety of distinct chemical environments within the micellar delivery system.70 These results can be used to rationally design a new linker between the hydrophobic and hydrophilic blocks.70
Numerous experimental studies have shown that the shape and size of drug delivery systems can influence the access of the drug to the target site.\textsuperscript{131,132} In a recent study, Peng \textit{et al.} combined all-atom MD and CG simulation to systematically investigate the size and shape of a polymer-drug conjugate: a 130-mer poly-$\gamma$-glutamyl-glutamate-paclitaxel conjugate (PGG\textsubscript{130}-paclitaxel).\textsuperscript{133} In this study, various fractions of paclitaxel were conjugated to six different positions of the PGG backbone making a total of eighteen different PGG\textsubscript{130}-paclitaxel conjugates. Due to limited computer resources, the MD simulations of PGG\textsubscript{130}-paclitaxel conjugates were first carried out in implicit water. Following 100 ns of MD simulation under these conditions, a CG model was used for the simulation of PGG\textsubscript{130}-paclitaxel conjugates in explicit solvent for an additional 800 ns. From an initial linear conformation at 0 ns, 89 % of PGG\textsubscript{130}-paclitaxel conjugates adopted a coil shape by 900 ns, indicating that the drug loading fraction and the position of conjugation have only a minor influence on the conformation of the conjugates.\textsuperscript{133} The coil shape was found to have several subtypes including angular, dense, extended, horseshoe, and dumbbell. Based on the simulation results, the size of PGG\textsubscript{130}-paclitaxel conjugates was 2 to 8 nm and was slightly affected by the drug loading fraction.\textsuperscript{133} A previous study on the delivery of paclitaxel using diblock copolymer micelles showed that wormlike and filamentous micelles exhibit prolonged circulation half-lives in comparison to spherical micelles.\textsuperscript{131} Further, discoidal particles seem to have relatively greater tendency to accumulate at tumors in comparison to spherical particles.\textsuperscript{132} Peng \textit{et al.} postulated that the PGG\textsubscript{130}-paclitaxel conjugates with various coil subtypes are structurally similar to the shapes of the wormlike, filamentous micelles, and discoidal
particles. Therefore, they proposed that the investigated PGG<sub>130</sub>-PTX conjugates may have a relatively long circulation half-life \textit{in vivo} and affinity for tumors.

Water molecules have a significant influence on the conformation and aggregation morphology of many delivery materials. In many cases, simulations must therefore be conducted in the presence of explicit molecular water in order to obtain results that are comparable to experiment. Computational acceleration that is obtained \textit{via} implicit water models may result in inaccurate simulation results as reviewed elsewhere.

1.4.4. Star-shaped block copolymer micelles

Unimolecular micelles are inherently stable to dilution and may be prepared to be monodisperse and of a smaller size than most multimolecular systems. Star-shaped block copolymers represent a possible material for unimolecular micelles, provided the molecules can be engineered to avoid self-aggregation.

Recently, MD simulations were employed to investigate the morphology of blending star and linear block copolymers micelles. For example, Xin \textit{et al.} used dissipative participle dynamics (DPD) simulations to investigate the morphology of multicompartent micelles formed by binary mixtures of star A<sub>a</sub>B<sub>b</sub>C<sub>c</sub> and linear B<sub>b</sub>A<sub>a</sub>C<sub>c</sub> triblock copolymers. In this study, polyethylethylene, PEO, and poly(perfluoropropane oxide) are polymers A<sub>a</sub>, B<sub>b</sub>, and C<sub>c</sub>, respectively, in which a, b, and c represent the block lengths. Here, the DPD model reproduced the experimental morphology of diblock copolymers (spherical micelles), triblock star copolymers (wormlike micelles), and blends of diblock and triblock star copolymers (hamburger
shaped micelles). Further, the morphologies of blended linear and star triblock copolymers were systematically investigated with various blending ratios and procedures. Various new morphologies were obtained, including toroidal multicompartment micelles with ring/cogwheel cores and so-called sphere-on-onion micelles. Finally, the mechanisms of formation of these morphologies were also identified.

Molecular simulations are the most suitable techniques for quantitative measurement of interactions at the molecular level and for the rational design of new drug delivery materials.

1.4.5. Dendrimers

Dendrimers are branched macromolecules in which each level of branching is classified as a generation. Generally, dendrimeric polymers developed for drug delivery have a multifunctional hydrophobic inner core that is conjugated to outer hydrophilic moieties, such as PEG. Similar to star-shaped block copolymers, dendrimer micelles can be rationally designed to be unimeric. Dendrimeric polymers are promising drug delivery materials since they possess narrow polydispersity and can be designed with many combinations of size, shape and surface chemistry.

For the past decade, polyamidoamine-PEG conjugates (PAMAM-PEG) have been investigated for biomedical applications. Lee and Larson rationally designed stable PAMAM dendrimer-PEG conjugates using CG simulations based on the MARTINI parameterization. In this study, a total of eleven dendrimers were simulated in CG water and counter ions. Systems included single dendrimers of generation 3 to 5 (G3 to G5)
and dendrimers conjugated to 0.55 kDa or 5.0 kDa PEG blocks. The simulated Rg of dendrimer-PEG conjugates with 30, 60, and 88 arms of 5.0-kDa PEG (G3P5000-30, G4P5000-60, G5P5000-88) agreed well with the Rg values obtained from neutron scattering for dendrimer-conjugates with similar MW. The possibility of aggregation of the dendrimer-PEG conjugates was studied by simulating two G4 dendrimer-PEG conjugates with sixty arms of 0.55-kDa or 5-kDa PEG (G4P5000-60, G4P550-60) for a total of 400 ns. After 250 ns of simulation, two G4P5000-60 conjugates migrated apart and retained a distance of 18 nm between centers. A similar result was obtained for two G4P550-60 conjugates. This result contradicts the experimental study by Yang et al. which suggests that interpenetration of the PEG arms from two adjacent dendrimer-conjugates may exist when the MW of PEG increase from 2 to 5 kDa. Nevertheless, based on these results, the authors proposed that 5-kDa PEG can act as a stabilizer for the PAMAM G4 dendrimer.

Although CG methods are unable to provide information on interactions at the atomistic level, such as hydrogen bonding interactions, they provide excellent insight into large systems such as those investigating the aggregation of complex materials.

1.5. Conclusions and Outlook

Analytical approaches are useful for fast pre-screening during the development of delivery systems and drug derivatives. Available tools include group contribution methods for the calculation of SPs and lipophilicity, and statistical methods such as FH pairwise interactions. However, there are errors associated with the theoretical calculation of SP, logP and $\chi_{FH}$, especially when comparing compounds with different chemical
properties. Nevertheless, these theoretical models are suitable for guiding early material design.

In contrast, molecular simulations produce more reliable results. Molecular simulations are, however, more computationally expensive and are thus more suitable for re-scoring materials that were highly ranked by more rapid theoretical screening methods. Molecular simulations are also capable of investigating and quantifying molecular structures and interactions within nanotechnologies. Further, molecular simulations can be used to evaluate performance-related properties of drug delivery systems, including the size and conformation of nanoparticles.

This chapter discusses many publications that apply a range of theoretical and experimental methods. In many cases, however, systematic studies that evaluate and compare methodological options are unavailable. Further, very few studies test the predictive abilities of the models. As well likely problems associated with theoretical methods include over fitting and underreporting of theoretical predictions that do not match experimental evaluations. In many studies, simulation systems were simplified via removing explicit water or using an implicit water model in order to accelerate the simulation. It is, however, unclear if these simulations are capable of representing the correct equilibrium distribution of conformations as they would exist in explicit water. Here again, systematic methodological evaluations are lacking.

In comparison to atomistic models, simulations with CG models can sample much larger systems for much longer times and are excellent methods for investing aggregation morphologies and other thermodynamic quantities that are slow to converge. However, CG models fail to capture important atomistic effects such as hydrogen bonding.
interactions and may therefore require re-parameterization for each system of interest. Combinations of atomistic and CG simulation can be used for guiding the design of new delivery materials such as dendrimers and star shaped block copolymers. To further quantify the propensity toward aggregation, efficient generalized-ensemble simulation algorithms can be used to construct the free energy profile governing the association of these materials.

The continuing advances in computer performance are allowing atomistic simulations of macromolecules over ever-growing timescales. Concurrently, analytical methods can be applied to ever larger library of drugs and materials. This promises a bright future for the development of drug delivery materials.

1.6. Thesis Research Objectives

Overall, the objective of the work described in this thesis is to apply theoretical methods to enhance the performance of hydrophobic drug formulations for intravenous administration. This work highlights the utility of computation methods for guiding the design of drug formulations and enhancing material-drug compatibility and stability, with specific application to nano-emulsions and polymer micelle formulations in aqueous solution. To this end, two complementary approaches are pursued:

1) Enhancing the drug’s solubility and loading efficiency in the formulation by (i) optimizing the pharmaceutical excipient (Scheme 1.3, top panel A) and (ii) modifying the drug (Scheme 1.3, bottom panel A). This strategy is pursued in Chapters 2 and 3.

2) Enhancing formulation stability by rationally designing unimolecular SCP micelles (Scheme 1.3, panel B). This strategy is pursued in Chapter 4.
In Chapter 2, all-atom MD simulation and semi-empirical methods are employed to predict the miscibility of the anti-cancer agent docetaxel in various pharmaceutical excipients. The solubilities and Flory-Huggins interaction parameters are calculated for docetaxel and various excipients including triglycerides, vitamin E, and β-caryophyllene (Scheme 1.3, panel A). The predicted values for solubility of DTX in good excipients (three different triglycerides and vitamin E) are within 2 to 6 % of the experimental values. Although the simulation method did not accurately predict the solubility of docetaxel in the poor excipient β-caryophyllene, the rank order of the solubility predictions is correct. Overall, these results suggest that simulation is an excellent method for ranking candidate materials and thus is useful for library enrichment.

In Chapter 3, a drug conjugation approach is used to further improve the stability and solubility of docetaxel in a triglyceride-based nano-emulsion (Scheme 1.3, bottom of panel A). Here, semi-emperical models are used to direct the chemical modification of docetaxel. The predicted optimal docetaxel conjugates are synthesized by direct attachment of formulation-compatible lauroyl moieties to enhance the solubility in the internal phase of a nano-emulsion formulation. Experimental results confirm that the conjugates are significantly more soluble in the oil, with high drug entrapment efficiencies (up to 97%) and a high drug loading capacity (5.7 % w/w) for the docetaxel conjugates. Overall, the computational methods applied in this study can be used to direct drug modification with great efficiency.

In Chapter 4, all-atom MD simulations are used to systematically investigate the solution properties of differently composed SCPs. The goal is to rationally design a stable unimolecular SCP that, as a unimer, will not disassemble and leak drug upon the dilution
that accompanies intravenous injection (Scheme 1.3, panel B). Importantly, the atomistic resolution of the simulations explicitly treats the physical basis of molecular self-aggregation, including the interactions that underlie the hydrophobic effect. The simulations are therefore capable of delineating which compositions will avoid self-aggregation and have the potential to form unimeric delivery systems. In this work, the propensity for aggregation of SCPs into multimolecular micelles is correlated with the partial hydration of the hydrophobic core of unimers. The SCPs with a hydrophobic PCL core $\leq 2$ kDa and hydrophilic PEG blocks approaching 14.6 kDa per arm are predicted to form unimolecular micelles that remain unimeric at high concentrations. In addition, the systematic methodology developed in this study is applicable to the design of arbitrarily composed polymers for which the principle requirement is the protection of a hydrophobic surface from aqueous solution.
**Scheme 1.3:** Enhancing hydrophobic drug formulations for intravenous administration by: (A) improving drug solubility through optimization of pharmaceutical solvents for a drug (top), and conjugation of drug to formulation-compatible moieties (bottom). (B) Quantification of hydration of the loadable core of these star copolymers provides fundamental insight into the thermodynamic basis of aggregation (top) and open the way towards the rational design of novel unimolecular star copolymers (bottom).
Chapter 2: Predicting the Solubility of the Anti-Cancer Agent Docetaxel in Small Molecule Excipients using Computational Methods

The work described in this chapter has been published in the following reference:

**Abstract:** The purpose of this study is to develop an in silico model that provides an accurate prediction of the relative solubility of the lipophilic anticancer agent docetaxel in various excipients. The *in silico* solubility of docetaxel in the excipients was estimated by means of the solubility ($\delta$) and Flory-Huggins interaction ($\chi_{FH}$) parameters. The $\delta$ values of docetaxel and excipients were calculated using semi-empirical methods and molecular dynamics (MD) simulations. Cerius$^2$ software and COMPASS force-field were employed for the MD simulations. The $\chi_{FH}$ values for the binary mixtures of docetaxel and excipient were also estimated by MD simulations. The values obtained from the MD simulations for the solubility of docetaxel in the various excipients were in good agreement with the experimentally determined values. The simulated values for solubility of docetaxel in tributyrin, tricapron and vitamin E were within 2 to 6 % of the experimental values. MD simulations predicted docetaxel to be insoluble in $\beta$-caryophyllene and this result correlated well with experimental studies. Overall, the MD model proved to be a reliable tool for selecting suitable excipients for the solubilization of docetaxel.
2.1. Introduction

Drug formulations are employed as a means to improve the solubility, stability, toxicity and/or efficacy of a drug.\textsuperscript{142-144} The formulations are most often formed from excipients such as phospholipids, medium-chain triglycerides and polymers.\textsuperscript{33, 106, 144, 145} The physico-chemical characteristics of the drug-excipient blend are known to determine the properties and performance of the formulation.\textsuperscript{38, 146} Specifically, the drug loading and retention properties of the formulation are largely influenced by the solubility of the drug in the excipient or miscibility of the drug-excipient blend as well as the presence of specific interactions between the drug and the excipient.\textsuperscript{77, 147}

To date, the development of most drug formulations proceeds largely by trial and error with no clear method of predicting which excipient or material is most appropriate. In this way, the selection of a suitable excipient or material can be a time consuming and expensive endeavour. Molecular simulation of drug-excipient mixtures provides an attractive alternative for predicting the solubility of drugs in excipients.

Recently, atomistic simulations were shown to provide an accurate prediction of the compatibility between polymer-small molecule\textsuperscript{148-152} and polymer-polymer blends\textsuperscript{153, 154}. In the area of drug formulation and delivery, molecular simulation has mostly been employed to predict the rate of diffusion of a drug in a matrix as a means to elucidate the mechanism of drug release.\textsuperscript{143, 151, 155} For example, Jacobson investigated the diffusion of drugs in a Duro-Tak polymer matrix (i.e. pressure-sensitive adhesive acrylic polymers used in transdermal drug delivery), in the presence and absence of an external force, using molecular dynamics (MD) simulations with the Discover module (InsightII software).\textsuperscript{155} The diffusion coefficients that were obtained for various drugs (e.g. nicotine,
estradiol, sodium salicylate) in the polymer matrix were related to the polymer-drug interactions and the free volume of the polymer.\textsuperscript{155} In a few other cases, molecular simulations have been employed to predict other properties of drug delivery systems (e.g. morphology, stability and interactions) at the molecular and coarse-grain levels.\textsuperscript{156-158} For example, Poupaerta and Couvreur calculated the interaction energy between the anticancer drug doxorubicin and \textit{n}-butyl polycyanoacrylate using molecular simulations.\textsuperscript{152} From the calculated interaction energy, it was possible to identify the site of interaction for the drug and the functional groups of the polymer that are involved in the interaction.\textsuperscript{152}

In this study, we report an \textit{in silico} method for predicting the solubility of the anticancer agent docetaxel (DTX) in excipients using full atomistic simulation. DTX is a member of the taxoid family and is approved for use in the treatment of prostate, gastric, lung, breast and head and neck cancers.\textsuperscript{159-166} The primary objective of this research was to evaluate the accuracy and reliability of various \textit{in silico} methods as a means to select suitable excipients for development of an emulsion formulation of DTX. The ability of an excipient to solubilize DTX may be expressed in terms of the Hildebrand solubility parameter ($\delta_{\text{HIL}}$) which describes the behavior of apolar and non-interacting liquids.\textsuperscript{54} However, the presence of hydrogen-bonding interactions can also influence the solubility of compounds.\textsuperscript{77,167} Therefore, in order to more accurately describe the behavior of the mixtures with consideration given to the polar effects and hydrogen-bonding interactions, the Hansen solubility parameter ($\delta_{\text{HAN}}$) and Flory-Huggins interaction parameter ($\chi_{\text{FH}}$) were calculated\textsuperscript{168} Semi-empirical methods and MD simulations were employed to evaluate the solubility parameters ($\delta$) for DTX and excipients.\textsuperscript{169-171} Furthermore, MD
simulations were used to calculate $\chi_{FH}$ for drug and excipient pairs. The values obtained for $\chi_{FH}$ were used to predict the solubility of DTX in the excipients$^{171}$

2.2. Materials and Experimental Methods

2.2.1. Materials

Anhydrous DTX (99.8%) was obtained from Sai Life Sciences (Hyderabad, India). Tricaprylin (90%), tricaprin ($\geq 99$%), tributyryl ($\geq 98$%), vitamin E ($\geq 97$%), and β-caryophyllene ($\geq 80$%) and high performance liquid chromatography (HPLC) grade solvents were purchased from Sigma Aldrich (Oakville, ON, Canada) and used as received.

2.2.2. Evaluation of solubility of DTX in excipients

The solubility of DTX was evaluated in tricaprylin, vitamin E, tricaprin, tributyryl, and β-caryophyllene at room temperature using the method established by Higuchi and Connors with slight modification$^{172-175}$ A rough estimate of the solubility of DTX in each excipient was first obtained by preparing a series of DTX-excipient mixtures that varied systematically in terms of the initial weight percentage of DTX. Specifically, aliquots of a stock solution of DTX dissolved in ethanol were added to glass vials that were then dried under nitrogen and in a vacuum oven overnight to produce DTX films. The excipients were then added to the DTX films and the mixtures were vortexed and stirred for eight hours at room temperature. DTX and vitamin E were allowed to stir for 24 hours due to the viscous nature of these mixtures (i.e. 24 hours). Longer stirring times (i.e. 48 hours for DTX-vitamin E and 24 hours for all other DTX-excipient mixtures) were tried
and found to yield comparable values for equilibrium solubility. Aliquots of the DTX-excipient mixtures were transferred to eppendorf tubes and centrifuged for 50 min at 20,000 g (Eppendorf 5804R, Eppendorf Inc., Hamburg, Germany) in order to separate the solution (i.e. supernatant) from the un-dissolved fraction of DTX (i.e. precipitate). The supernatant was collected and the concentration of dissolved drug was determined by HPLC analysis. Once an estimate of the solubility of DTX in each excipient had been obtained the equilibrium solubility was measured by mixing each excipient with excess DTX (n = 5). The mixtures of excess DTX and excipient were stirred and processed as outlined above.

2.2.3. HPLC analysis

DTX was extracted from the excipients using a previously reported method with slight modifications.\textsuperscript{176-178} The mixtures of DTX in excipients were added to an acetonitrile : water : hexane mixture (45:5:50 v/v/v) and processed for HPLC analysis. The extraction efficiency of DTX was found to be 94 ± 4 % (n = 6) from tributyrin, 88 ± 5% (n = 6) from tricaprolin, 95 ± 3% (n = 6) from tricaprylin, 95 ± 3% (n = 6) from vitamin E, and 59 ± 9% (n = 6) from β-caryophyllene.

The DTX concentration was measured using an HPLC (Perkin-Elmer series 200 Liquid Chromatograph, PerkinElmer Inc, Wellesley, MA) equipped with a Perkin-Elmer 785A UV/VIS detector, Perkin-Elmer Advanced LC sample processor and an XTerra C\textsubscript{18} reverse-phase column (particle size, 5 µm) of dimensions 4.6 x 250 mm (Waters Inc., Milford, MA). The concentration of DTX was detected at a wavelength of 227 nm. For DTX in tricaprylin, tricaprolin, tributyrin or β-caryophyllene, the mobile phase
(acetonitrile and water, 53:47 v/v) was eluted isocratically. For DTX in vitamin E, an HPLC gradient elution method was employed. Specifically, a mobile phase of acetonitrile : water (53:47 v/v) was used for the first 15 min to elute DTX followed by a mixture of acetonitrile : water : THF (53:22:25 v/v/v) for 6 min to elute vitamin E. Standard curves were constructed for DTX in each excipient and a linear range was obtained from 5.0 to 120.0 μg/mL DTX for all excipients. The retention time of DTX was 8 min at a flow rate of 1.0 mL/min.

2.3. Computational Methodology

2.3.1 Calculation of solubility parameters using group contribution methods and C²-Synthia module

In this study, the δ_HIL and δ_HAN values were used to quickly gain an estimate of the solubility of DTX in excipients based on the chemical structures of the molecules (Table 2.1). The semi-empirical methods, including group contribution methods (GCM) and C²-Synthia module from Cerius² software, were used to calculate the δ_HIL and δ_HAN by applying the Fedors and Hoftyzer-Van Krevelen approaches, respectively. The δ_HIL is defined as the square root of the cohesive energy density (CED), which is the heat or energy of vaporization of a material per unit volume in the amorphous state at room temperature. The Fedors method for determination of the δ_HIL (δ_HIL-GCM and δ_HIL-Syn) involves summation of the cohesive energy (E_coh) and volume (V) contributions from the individual functional groups within the molecule. In comparison, Hoftyzer-Van Krevelen’s method for determination of the δ_HAN (δ_HAN-GCM and δ_HAN-Syn) takes into account the different types of intermolecular forces.
Therefore, the $\delta_{\text{HAN}}$ (eq 2.1) is obtained from the partial solubility parameters (i.e. $\delta_d$, $\delta_p$, and $\delta_h$, eqs 2.2 – 2.4) which are calculated from values assigned to specific functional groups for dispersion ($F_d$), dipole-dipole ($F_{pi}$), hydrogen-bonding ($F_{hi}$) interactions as well as volume ($V$).

$$\delta_{\text{HAN}} = \sqrt{(\delta_d^2 + \delta_p^2 + \delta_h^2)} \quad (2.1)$$

$$\delta_d = \frac{\sum F_d}{V} \quad (2.2)$$

$$\delta_p = \sqrt{\frac{\sum F_{pi}^2}{V}} \quad (2.3)$$

$$\delta_h = \sqrt{\frac{\sum F_{hi}}{V}} \quad (2.4)$$

Theoretically, the smaller the difference between the values for $\delta$ of the solute and solvent, the greater the solubility of the solute in the solvent.\textsuperscript{54, 88, 180, 181} It has been reported that values for $\Delta\delta$ of less than 3.7 (J/cm\textsuperscript{3})\textsuperscript{1/2} for a solute-solvent pair indicate that the solute has a good solubility in the solvent.\textsuperscript{78}
Table 2.1: Chemical structures and properties of drugs and excipients.

<table>
<thead>
<tr>
<th>Drugs/Excipients</th>
<th>Molecular Weight (g/mol)</th>
<th>V\textsubscript{Molar} \textsuperscript{a} (cm\textsuperscript{3}/mol)</th>
<th>Density\textsuperscript{a} (g/cm\textsuperscript{3})</th>
<th>MP/BP (°C)</th>
<th>Water solubility [μg/mL]</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>807.9</td>
<td>664.4</td>
<td>1.216</td>
<td>MP: 232 \textsuperscript{b}</td>
<td>5.0 – 6.0 (78)</td>
<td>White crystalline powder</td>
</tr>
<tr>
<td>R\textsubscript{1} = H, R\textsubscript{2} = O–C(CH\textsubscript{3})\textsubscript{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>853.9</td>
<td>688.3</td>
<td>1.235</td>
<td>MP: 223 (70)</td>
<td>0.30 (78)</td>
<td>White crystalline powder</td>
</tr>
<tr>
<td>R\textsubscript{1} = C=CH\textsubscript{3}, R\textsubscript{2} = O\textsubscript{–}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E (d-α-Tocopherol)</td>
<td>430.7</td>
<td>453.4</td>
<td>0.950</td>
<td>MP: 2.5 - 3.5 BP: 200 - 220 \textsuperscript{b}</td>
<td>insoluble</td>
<td>Very faint yellow, clear viscous liquid</td>
</tr>
<tr>
<td>Tricaprylin (C\textsubscript{8}:0)</td>
<td>470.7</td>
<td>492.4</td>
<td>0.953</td>
<td>MP: 9 - 10 \textsuperscript{b} BP: 233 \textsuperscript{b}</td>
<td>insoluble</td>
<td>Colourless, clear liquid</td>
</tr>
<tr>
<td>Tricaprin (C\textsubscript{6}:0)</td>
<td>386.5</td>
<td>394.4</td>
<td>0.980</td>
<td>NA</td>
<td>0.45 (5)</td>
<td>Colourless, clear liquid</td>
</tr>
<tr>
<td>Tributyrin (C\textsubscript{4}:0)</td>
<td>302.4</td>
<td>293.0</td>
<td>1.032</td>
<td>BP: 129 - 131 \textsuperscript{b}</td>
<td>133.4 (5)</td>
<td>Colourless, clear liquid</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>204.3</td>
<td>226.5</td>
<td>0.902</td>
<td>BP: 262 - 264 \textsuperscript{b}</td>
<td>insoluble</td>
<td>Colourless, clear liquid</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Molar volumes (V\textsubscript{Molar}) of excipients were calculated from the experimental density and molecular weight of the excipients. \textsuperscript{b} V\textsubscript{Molar} and density of drugs were obtained by using C⁲-Synthia module. (b) MP/BP: melting point/boiling point were obtained from the material safety data sheet.
2.3.2. Calculation of solubility parameters using MD simulation

MD is a force-field based simulation method that is used to calculate the behavior of molecules in a time dependent manner.\textsuperscript{182, 183} MD takes into account the molecular motions (e.g. simple vibrations, bond stretching and angle bending) that occur within a system.\textsuperscript{182} MD simulations were used to calculate the $\delta_{\text{HIL}}$ values ($\delta_{\text{HIL-MD}}$) of DTX and excipients as well as the $\chi_{FH}$ for the DTX-excipient pairs. The Ewald method with application of the COMPASS (Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies)\textsuperscript{184} force-field was used to calculate the Coulombic and attractive van der Waals (vdW) interactions.\textsuperscript{185} For the COMPASS force-field, the total energy ($E_{\text{total}}$) of a system is represented by the sum of the valence interactions ($E_{\text{valence}}$), non-bonding interactions ($E_{\text{non\_bond}}$) and the cross-coupling term ($E_{\text{crossterm}}$):\textsuperscript{184, 185}

\begin{equation}
E_{\text{total}} = E_{\text{valence}} + E_{\text{crossterm}} + E_{\text{non\_bond}}
\end{equation}

The $E_{\text{valence}}$ consists of bond stretching ($E_{\text{bond}}$), angle energy ($E_{\text{angle}}$), torsion angle rotations ($E_{\text{torsion}}$), out-of-plane ($E_{\text{oop}}$) and Urey-Bradley interactions ($E_{\text{UB}}$):\textsuperscript{184}

\begin{equation}
E_{\text{valence}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{torsion}} + E_{\text{oop}} + E_{\text{UB}}
\end{equation}

The $E_{\text{crossterm}}$ includes the following interaction energies: the stretch-stretch ($E_{\text{bond-bond}}$), stretch-bend ($E_{\text{bond-angle}}$), bend-bend ($E_{\text{angle-angle}}$), stretch-torsion ($E_{\text{central-bond-torsion}}$ and $E_{\text{terminal-bond-torsion}}$), bend-torsion ($E_{\text{angle-torsion}}$) and bend-bend-torsion ($E_{\text{angle-angle-torsion}}$):\textsuperscript{184, 186}

\begin{equation}
E_{\text{crossterm}} = E_{\text{bond-bond}} + E_{\text{bond-angle}} + E_{\text{angle-angle}} + E_{\text{central-bond-torsion}} + E_{\text{terminal-bond-torsion}} + E_{\text{angle-torsion}} + E_{\text{angle-angle-torsion}}
\end{equation}

The interaction energy between non-bond atoms is comprised of the vdW interaction energy ($E_{\text{vdW}}$) and the electrostatic interaction energy ($E_{\text{Coulomb}}$), which is
calculated by a Coulombic function based on the partial charges of the atoms in the system.\textsuperscript{185}

\[ E_{\text{non\_bond}} = E_{\text{vdW}} + E_{\text{Coulomb}} \]  

(2.8)

The difference in the \( E_{\text{total}} \) of the molecules in the vacuum state \( (E_{\text{vac}}) \) and the amorphous state \( (E_{\text{bulk}}) \) can be employed to calculate the \( \delta_{\text{HIL}} \) which may be defined by the following equation:\textsuperscript{153}

\[ \delta_{\text{HIL}} = \sqrt{\frac{E_{\text{coh}}}{V}} = \sqrt{\frac{(E_{\text{vac}} - E_{\text{bulk}})C}{V}} = \sqrt{\text{CED}} \]  

(2.9)

where \( V \) is the volume of the periodic cell in cubic angstroms. The units for \( E_{\text{coh}} \) obtained from the MD simulation are expressed in \( \text{(kcal/(mol\AA^3)}) \), which are then converted to \( J/cm^3 \) using the converting factor \( C \) that is calculated as follows:

\[ C = \frac{kcal}{mol \AA^3} = \frac{4184 J}{N_A \times 1 \times 10^{-24} cm^3} = \frac{4184 J}{(6.022 \times 10^{23})(1 \times 10^{-24} cm^3)} = 6947.86 \frac{J}{cm^3} \]

2.3.3. Calculation of Flory-Huggins interaction parameters and prediction of solubility using MD simulation

Theoretically, at a specific temperature, the solubilization of a solute in a solvent \( (i.e. \) one phase solution) is attributed to favourable entropic \( (i.e. \) positive) and/or enthalpic \( (i.e. \) negative) contributions that are reflected by either a small positive or a negative energy of mixing \( (\Delta E_{\text{mix}}) \). \( \Delta E_{\text{mix}} \) for DTX-excipient mixtures was calculated from the CED of the pure DTX \( ([E_{\text{coh}}/V]_{\text{DTX}}) \), pure excipient \( ([E_{\text{coh}}/V]_{\text{EXC}}) \) and DTX-excipient mixtures \( ([E_{\text{coh}}/V]_{\text{DTX-EXC}}) \) using eq 2.10.\textsuperscript{153}
\[ \Delta E_{\text{mix}} = \phi_{\text{DTX}} \left( \frac{E_{\text{coh}}}{V} \right)_{\text{DTX}} + \phi_{\text{EXC}} \left( \frac{E_{\text{coh}}}{V} \right)_{\text{EXC}} - \left( \frac{E_{\text{coh}}}{V} \right)_{\text{DTX-EXC}} \]  \hspace{1cm} (2.10)

where \( \phi_{\text{DTX}} \) and \( \phi_{\text{EXC}} \) are the volume fractions of DTX and excipient in the binary mixed system, respectively. \( E_{\text{coh}} \) is the cohesive energy and \( V \) is the total volume of the system. The volume fraction (\( \phi \)) can be defined as \( \phi_i = \left( \frac{n_i V_i}{V} \right) \), where \( n_i \) is the number of moles and \( V_i \) is the volume of compound \( i \).\(^{187}\)

In this study, \( \chi_{\text{FH}} \) values were calculated using eq 2.11.\(^{81,82}\)

\[ \chi_{\text{FH}} = \frac{V_{\text{ref}}}{RT} \Delta E_{\text{mix}} \] \hspace{1cm} (2.11)

where \( V_{\text{ref}} \) is equal to the molar volume of the excipient (\( i.e. \) smaller molecule in the binary mixtures) that is obtained from the experimental density and molecular weight of the excipient. \( \chi_{\text{FH}} \) was originally introduced, in Flory-Huggins (FH) theory, to describe the interactions in polymer-solvent systems.\(^{45}\) FH theory is a lattice-based model that was put forth to characterize the thermodynamic behaviour of apolar polymer solutions.\(^{45}\) This theory was developed with the assumptions that the configuration of molecules within the system is completely random and that no specific interactions are created or destroyed upon mixing of the two components.\(^{45}\) The MD simulations performed in these studies are based on modified-FH theory that takes into account specific intermolecular interactions present between the two components within the mixture.\(^{82}\) This modified-FH theory also allows for evaluation of the concentration dependence of \( \chi_{\text{FH}} \).\(^{82}\)

The relationship between \( \chi_{\text{FH}} \) and the Gibbs energy of mixing (\( \Delta G_{\text{mix}} \)) is as follows:\(^{187,188}\)
\[
\Delta G_{\text{mix}} = RT \left[ n_1 \ln \phi_1 + n_2 \ln \phi_2 + n_1 \phi_2 \chi_{FH} \right]
\]  \hspace{1cm} (2.12)

where \( n_i \) and \( \phi_i \) are the number of moles and volume fraction of components 1 and 2, respectively. The first two terms are said to account for combinatorial entropy contributions while the third term is an enthalpic contribution.\(^{188}\) For the solution to be miscible \( \Delta G_{\text{mix}} \) must be negative, thus the enthalpic term must have a negative value or a positive value that is less in magnitude than that of the entropic contribution.\(^{188}\) Therefore, as \( \chi_{FH} \) approaches zero or negative values spontaneous mixing of the two component system is favored.

Theoretically, as outlined in Appendix A,\(^{45, 187, 189}\) phase separation of a polymer-solvent mixture begins to occur at the “critical” point when \( \chi_{FH} \) is equal to 0.5 (\( i.e. \) \( \chi_{FH, \text{crit}} \)). The “critical” values for \( \chi_{FH} \) can be expressed in term of volume fraction as shown in eq 2.13.\(^{45, 187, 189}\)

\[
\chi_{FH, \text{crit}} = \frac{1}{2} (1 - \phi_2)^2
\]  \hspace{1cm} (2.13)

Indeed, \( \chi_{FH} \) values of less than 0.5 have been obtained experimentally for a range of miscible polymer-solvent solutions.\(^{190, 191}\) In this study, the \( \chi_{FH} \) values obtained from MD simulations of each DTX-excipient mixture were plotted as a function of \( \phi_{\text{DTX}} \). The values for \( \phi_{\text{DTX}} \) at \( \chi_{FH} = 0.50 \) were then taken to be the predicted maximum solubility of DTX in each excipient.

### 2.3.4. Simulation methodology

MD simulations were performed using Cerius\(^2\) (C\(^2\)) software (version 4.6) from Accelrys Inc. (San Diego, CA.) on a Silicon Graphics OCTANE workstation (IRIX 6.5 operating system) that connects to an Onyx3800 supercomputer (44 MIPS processors) at
the Molecular Design and Information Technology Center (University of Toronto, Ontario). One processor was used per simulation of drug, excipient or mixtures of drug and excipient with an average processing time of approximately four months. The pure drug, excipients and their mixtures were built in periodic cells using the C²-Amorphous module. The density of the single-components and the mixed systems were defined according to the experimental density of excipients and the calculated density of DTX (i.e. obtained from C²-Synthia module) in order to mimic the experimental conditions. MD simulations were performed on the constructed periodic systems using C²-Dynamics module. The periodic cells of the pure component or binary mixtures contained approximately 5,000 atoms in a cell of 35 Å × 35 Å × 35 Å (as detailed further in Table 2.2). The non-bond cutoff for the vdW terms was applied with a cutoff distance of 8.5 Å.¹⁸⁴,¹⁸⁵ This optimal cutoff distance was less than half of the dimension of the cell.

Table 2.2: The properties of the pure component and the binary mixed systems of docetaxel (DTX) and excipients in the amorphous cells.

<table>
<thead>
<tr>
<th>Volume Fraction of DTX (ϕDTX)</th>
<th>Number of DTX : Tributyrin molecules</th>
<th>Number of DTX : Tricapronin molecules</th>
<th>Number of DTX : Tricaproin molecules</th>
<th>Number of DTX : Vitamin E molecules</th>
<th>Number of DTX : β-Caryophyllene molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>40: 0 (1.216)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000</td>
<td>0:94 (1.032)</td>
<td>0:68 (0.980)</td>
<td>0:55 (0.953)</td>
<td>0:58 (0.950)</td>
<td>0:114 (0.902)</td>
</tr>
<tr>
<td>0.025</td>
<td>1:88 (1.034)</td>
<td>1:66 (0.984)</td>
<td>1:53 (0.961)</td>
<td>1:57 (0.955)</td>
<td>1:114 (0.905)</td>
</tr>
<tr>
<td>0.050</td>
<td>2:86 (1.036)</td>
<td>2:64 (0.987)</td>
<td>2:51 (0.966)</td>
<td>2:56 (0.959)</td>
<td>2:111 (0.908)</td>
</tr>
<tr>
<td>0.075</td>
<td>3:84 (1.038)</td>
<td>3:62 (0.991)</td>
<td>3:50 (0.971)</td>
<td>3:54 (0.964)</td>
<td>3:109 (0.910)</td>
</tr>
<tr>
<td>0.100</td>
<td>4:82 (1.041)</td>
<td>4:61 (0.995)</td>
<td>4:49 (0.976)</td>
<td>4:53 (0.969)</td>
<td>4:106 (0.913)</td>
</tr>
</tbody>
</table>

Values in parentheses are the density (g/cm³) of the amorphous cells.
The simulation methodology for the pure and binary mixed systems is outlined in Figure 2.1. All of the periodic cells were constructed and analyzed to ensure a homogenous distribution of molecules in the periodic system. In addition, sufficient interaction between the two components (i.e. no “empty holes”) within the initial configuration of the binary system was also required for MD simulation.\textsuperscript{153} The optimal systems were minimized using the “Smart Minimizer” algorithms for 5,000 steps or until the maximum derivative (i.e. root-mean-square of the potential energy gradient) was less than 0.1 kcal/mol/Å.\textsuperscript{192} Following the energy minimization step, Nosé–Hoover constant-temperature, constant-volume ensemble (NVT) was applied to the system at 298 K.\textsuperscript{193,194} The time required to reach the equilibrium state depended on the size of the system and the molecular structure of the components.\textsuperscript{182}

\textbf{Figure 2.1:} Flowchart of approach taken for molecular dynamics simulations of the pure and binary mixed systems of docetaxel and excipient.
The general methodology for MD simulations includes two stages: equilibration and production (or data-collection).\textsuperscript{171} Initially, the velocities are randomly assigned to atoms in the model according to the Maxwell–Boltzmann distribution. A time step of 1 fs was used with a minimum runtime of 100 ps for the equilibration stage. For the production stage, the MD simulation time was 800 ps or until equilibrium was reached (\textit{i.e.} maximum 2200 ps). The configurations from MD simulations were saved every 1 ps to the trajectory file. Finally, the CED were evaluated over the last 50 ps of the MD simulations.\textsuperscript{185} In this study, $\phi_{\text{DTX}}$ in the various excipients ranged from 0.025 to 0.100. A typical example of the initial configuration of a periodic cell containing two DTX and 86 tributyrin molecules (\textit{i.e.} $\phi_{\text{DTX}} = 0.050$) is shown in Figure 2.2.

\textbf{Figure 2.2:} An amorphous cell containing 2 docetaxel and 86 tributyrin molecules. Tributyrin and docetaxel are shown by stick and space-filling representations, respectively.
2.4. Results and Discussion

2.4.1. Experimental solubility

The excipients selected for this research include vitamin E (*i.e.* d-α-tocopherol), tributyrin, tricaprin, tricaprylin and β-caryophyllene which are all generally recognized as safe and/or listed by the Food and Drug Administration for use as pharmaceutical or food additives (Table 2.1). As listed in Table 2.3, the experimental solubility of DTX in the various excipients ranged from 0.4 to 108 mg/mL with the solubility being highest in tributyrin. The solubility of DTX in the saturated, medium-chain triglycerides (i.e. tributyrin (C4:0), tricaprin (C6:0) and tricaprylin (C8:0)) increased with a decrease in the hydrocarbon chain length. These results are in agreement with a study by Kan et al. which found that the solubility of paclitaxel (PTX) in various triglycerides (i.e. tributyrin, tricaprin, tricaprylin and triacetin (C2:0)) increased as the length of the hydrocarbon chain of the triglyceride decreased. PTX is a clinically relevant analog of DTX and their structures vary in terms of the functional groups present at the C-10 and C-5’ positions, as shown in Table 2.1. It has been reported that in the presence of triglycerides, the intra- and intermolecular hydrogen-bonding between PTX molecules is replaced by hydrogen-bonding interactions between the drug and the triglyceride molecules. The solubility of PTX or DTX in triglycerides may be related to their potential to engage in hydrogen-bonding interactions with the carbonyl functional group of the triglyceride. Indeed, the number of moles of the carbonyl functional groups available to form hydrogen bonds with DTX was highest for tributyrin (1.13×10⁻³ mol) in comparison to tricaprin (0.85×10⁻³ mol) and tricaprylin (0.68×10⁻³ mol), per mL of triglyceride.
<table>
<thead>
<tr>
<th>Excipients</th>
<th>Experimental Solubility (mg/mL)(^a)</th>
<th>Simulated Solubility (mg/mL)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tributyrin</td>
<td>108 ± 1.8 (colourless, clear liquid)</td>
<td>114.4 (0.086)</td>
</tr>
<tr>
<td>Tricaprin</td>
<td>85.7 ± 2.0 (colourless, clear liquid)</td>
<td>88.7 (0.068)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>75.0 ± 1.8 (very faintly yellow, clear viscous liquid)</td>
<td>76.2 (0.059)</td>
</tr>
<tr>
<td>Tricaprylin</td>
<td>55.6 ± 2.2 (colourless, clear liquid)</td>
<td>65.3 (0.051)</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>0.43 ± 0.09 (colourless, clear liquid)</td>
<td>&lt; 31.2 (&lt;0.025)</td>
</tr>
</tbody>
</table>

(a) Values are the mean of five measurements; the physical appearance of the docetaxel-excipient solutions are described as in parentheses. (b) The simulated solubility was taken to be the volume fraction of docetaxel (values in parentheses) that corresponded to a value for the Flory-Huggins interaction parameter of 0.5 (Figure 2.4).

Table 2.3: Experimentally determined and simulated values for the solubility of docetaxel in the various excipients at room temperature.
The hydrogen-bonding interactions between DTX and these relatively hydrophobic excipients are intermolecular interactions that only act to enhance the solubility of the drug. It is postulated that the drug's solubility in these excipients is mostly attributed to dispersion and other vdw forces that are known to exist between hydrophobic drugs and hydrophobic excipients.\textsuperscript{201, 202}

2.4.2. \textit{In silico} solubility parameters for DTX and excipients

The solubility parameters of DTX ($\delta_{\text{DTX}}$) and excipients ($\delta_{\text{EXC}}$) were determined by \textit{in silico} methods as listed in Table 2.4. For each DTX-excipient pair, we calculated the difference between $\delta_{\text{DTX}}$ and $\delta_{\text{EXC}}$ values ($\Delta \delta$) obtained from GCM ($\Delta \delta_{\text{HIL-GCM}}$, $\Delta \delta_{\text{HAN-GCM}}$), $C^2$-Synthia module ($\Delta \delta_{\text{HIL-Syn}}$, $\Delta \delta_{\text{HAN-Syn}}$) and MD ($\Delta \delta_{\text{HIL-MD}}$) in order to screen for excipients that are most suitable for solubilization of DTX (Table 2.5). As discussed previously, a lower value for $\Delta \delta$ for a specific DTX-excipient pair should result in a higher solubility for DTX in that excipient. However, if one compound contains functional groups that are strongly polar and/or have hydrogen donor/acceptor capability while the other compound does not, the mixture may be immiscible even if the $\delta$ values of the two compounds are the same.\textsuperscript{88} This is due to the fact that the $\delta_{\text{HIL}}$ was initially proposed for only apolar, non-associating liquid (\textit{i.e.} solvent) systems.\textsuperscript{88} The ability of the computational methods to enable an accurate selection of the optimal excipient for DTX was verified by comparison with the experimentally determined values for solubility. According to values obtained for $\Delta \delta$ from the semi-empirical methods, the estimated solubility of DTX in the excipients decreased in the following order:

From $\Delta \delta_{\text{HAN-GCM}}$ values:
Vitamin E > Tributyrin > Tricaproin > β-Caryophyllene ≈ Tricaprylin

From $\Delta \delta_{\text{HIL-GCM}}$ and $\Delta \delta_{\text{HAN-Syn}}$ values:

Vitamin E > Tributyrin > Tricaproin > Tricaprylin > β-Caryophyllene

From $\Delta \delta_{\text{HIL-Syn}}$ values:

Tributyrin > Tricaproin > Vitamin E ≈ Tricaprylin > β-Caryophyllene

**Table 2.4**: Hildebrand and Hansen solubility parameters of docetaxel and excipients calculated using group contribution methods, $C^2$-Synthia module and molecular dynamics simulations at 298 K.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\delta_{\text{HAN-GCM}}^{a, b}$</th>
<th>$\delta_{\text{HIL-GCM}}^c$</th>
<th>$\delta_{\text{HAN-Syn}}^a$</th>
<th>$\delta_{\text{HIL-Syn}}^c$</th>
<th>$\delta_{\text{HIL-MD}}^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>27.14 (21.91, 3.51, 15.63)</td>
<td>28.28</td>
<td>23.88</td>
<td>24.26</td>
<td>20.17</td>
</tr>
<tr>
<td>Tributyrin</td>
<td>18.90 (16.54, 3.01, 8.62)</td>
<td>19.84</td>
<td>17.84</td>
<td>20.53</td>
<td>18.89</td>
</tr>
<tr>
<td>Tricaproin</td>
<td>18.33 (16.60, 2.24, 7.44)</td>
<td>19.27</td>
<td>17.57</td>
<td>19.77</td>
<td>18.13</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>19.50 (17.91, 1.59, 7.55)</td>
<td>20.08</td>
<td>19.29</td>
<td>19.30</td>
<td>17.81</td>
</tr>
<tr>
<td>Tricaprylin</td>
<td>18.00 (16.64, 1.78, 6.65)</td>
<td>18.93</td>
<td>17.41</td>
<td>19.31</td>
<td>17.76</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>18.03 (18.03, 0.00, 0.00)</td>
<td>18.37</td>
<td>17.03</td>
<td>17.87</td>
<td>16.86</td>
</tr>
</tbody>
</table>

The values for $\delta$ are expressed in (J/cm$^3$)$^{1/2}$; (a) Hansen solubility parameters obtained using group contribution methods ($\delta_{\text{HAN-GCM}}$) and $C^2$-Synthia module ($\delta_{\text{HAN-Syn}}$); (b) values in parentheses are Hansen partial solubility parameters including $\delta_d$, $\delta_p$ and $\delta_h$, respectively; (c) Hildebrand solubility parameters obtained using group contribution methods ($\delta_{\text{HIL-GCM}}$), $C^2$-Synthia module ($\delta_{\text{HIL-Syn}}$), and molecular dynamics simulation ($\delta_{\text{HIL-MD}}$).
Calculations using the GCM and C²-Synthia module accurately predicted the relative degree of solubility of DTX in the triglycerides (*i.e.* tributyrin, tricaproin, tricaprylin) as the ranking was in agreement with the experimental results. Furthermore, the relative degree of solubility predicted from the $\Delta \delta_{\text{HIL-Syn}}$ was similar to the experimental results with the exception that DTX was predicted to be equally soluble in vitamin E and tricaprylin. The predicted solubility, according to the values of $\Delta \delta_{\text{HAN-GCM}}$, $\Delta \delta_{\text{HIL-GCM}}$ and $\Delta \delta_{\text{HAN-Syn}}$ for the DTX-vitamin E and DTX-β-caryophyllene pairs, disagreed with the experimental results. In contrast, the values obtained for $\Delta \delta_{\text{HIL-MD}}$ correctly predicted the solubility of DTX in all of the excipients investigated. From the MD results, the solubility of DTX in the various excipients was predicted to decrease in the following order:

Tributyrin > Tricaproin > Vitamin E > Tricaprylin > β-Caryophyllene

The $\delta_{\text{DTX}}$ values calculated by GCM method ($\delta_{\text{HIL-GCM}}, \delta_{\text{HAN-GCM}}$) were significantly larger than the values obtained from C²-Synthia module ($\delta_{\text{HIL-Syn}}, \delta_{\text{HAN-Syn}}$) and MD ($\delta_{\text{HIL-MD}}$). Hence, for each of the DTX-excipient pairs investigated, large $\Delta \delta_{\text{HIL-GCM}}$ and $\Delta \delta_{\text{HAN-GCM}}$ values (> 7.6 (J/cm³)¹/²) were obtained (Table 2.5). Therefore, the GCM method was considered less accurate for calculating $\delta$ values for large and bulky molecules such as DTX. Nevertheless, the $\delta_{\text{HAN-GCM}}$ values provide details of the interactions that contribute to the total solubility parameter. Given that β-caryophyllene is an apolar solvent, the contributions of polar groups ($\delta_p$) and hydrogen-bonding ($\delta_h$) interactions to the $\delta_{\text{HAN-GCM}}$ value for β-caryophyllene were zero which explains the poor experimental solubility of DTX in β-caryophyllene (Table 2.3 and 2.4). When
considering only the $\delta_h$ value, which represents the hydrogen-bonding donor/acceptor capability of the excipients, tributyrin had the highest $\delta_h$ value (8.6 (J/cm$^3$)$^{1/2}$) followed by tricaproin (7.4 (J/cm$^3$)$^{1/2}$) and then tricaprylin (6.6 (J/cm$^3$)$^{1/2}$). The $\delta_h$ value for vitamin E (7.6 (J/cm$^3$)$^{1/2}$) was slightly larger than tricaproin indicating that vitamin E has the ability to form hydrogen-bonding interactions with DTX. The hydroxyl group within the chemical structure for vitamin E may act as a hydrogen acceptor and donor. Thus, as mentioned previously, the solubility of DTX in the various excipients may be related to the extent of hydrogen-bonding between the drug and excipient. Similar observations were made in studies evaluating the solubility of ibuprofen in various excipients (i.e. poloxamer 188, maltose, sorbitol) in solid dispersion formulations $^{87}$.

**Table 2.5**: The difference between the solubility parameters of docetaxel and excipients.

<table>
<thead>
<tr>
<th>Docetaxel/excipient</th>
<th>$\Delta\delta_{\text{HAN-GCM}}^a$</th>
<th>$\Delta\delta_{\text{HIL-GCM}}^b$</th>
<th>$\Delta\delta_{\text{HIL-Syn}}^b$</th>
<th>$\Delta\delta_{\text{HIL-MD}}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel/Tributyrin</td>
<td>8.24</td>
<td>8.44</td>
<td>6.04</td>
<td>3.73</td>
</tr>
<tr>
<td>Docetaxel/Tricaproin</td>
<td>8.81</td>
<td>9.01</td>
<td>6.31</td>
<td>4.49</td>
</tr>
<tr>
<td>Docetaxel/Vitamin E</td>
<td>7.64</td>
<td>8.20</td>
<td>4.59</td>
<td>4.96</td>
</tr>
<tr>
<td>Docetaxel/Tricaprylin</td>
<td>9.14</td>
<td>9.35</td>
<td>6.47</td>
<td>4.95</td>
</tr>
<tr>
<td>Docetaxel/$\beta$-Caryophyllene</td>
<td>9.11</td>
<td>9.91</td>
<td>6.85</td>
<td>6.39</td>
</tr>
</tbody>
</table>

The values for $\Delta\delta$ are expressed in (J/cm$^3$)$^{1/2}$; (a) calculated values using Hansen solubility parameters, obtained from group contribution methods ($\Delta\delta_{\text{HAN-GCM}}$) and C$^2$-Synthia module ($\Delta\delta_{\text{HAN-Syn}}$); (b) calculated values using Hildebrand solubility parameters obtained from group contribution methods ($\Delta\delta_{\text{HIL-GCM}}$), C$^2$-Synthia module ($\Delta\delta_{\text{HIL-Syn}}$) and molecular dynamics simulation ($\Delta\delta_{\text{HIL-MD}}$).
In excipients that DTX was found experimentally to have good solubility (Table 2.3) \(i.e.\) the solubility of DTX in excipient was greater than 50 mg/mL, \(\Delta \delta\) values of 3.7 - 6.5 \((J/cm^3)^{1/2}\) and 1.3 - 2.4 \((J/cm^3)^{1/2}\) were obtained from the \(C^2\)-Synthia module and MD simulations, respectively. For DTX in \(\beta\)-caryophyllene, values of 6.9 \((J/cm^3)^{1/2}\) and 6.4 \((J/cm^3)^{1/2}\) were obtained for \(\Delta \delta_{HAN-Syn}\) and \(\Delta \delta_{HIL-Syn}\), respectively; whereas, a \(\Delta \delta_{HIL-MD}\) value of 3.3 \((J/cm^3)^{1/2}\) was obtained from MD simulations. The values for \(\Delta \delta\) obtained from \(C^2\)-Synthia and MD models were relatively low \(i.e.\) less than 3.7 \((J/cm^3)^{1/2}\) for the \(\beta\)-caryophyllene and DTX pair. Yet, from consideration of the relative ranking of the excipients and the chemical structure of \(\beta\)-caryophyllene, it may be deduced that the solubility of DTX in \(\beta\)-caryophyllene is poor. In comparison, DTX and the other excipients investigated can interact as they possess polar and hydrogen-bonding groups. These observations were further confirmed from the values for the Hansen partial solubility parameters for polar and hydrogen-bonding contributions \(i.e.\) \(\delta_p\) and \(\delta_h\) as discussed above.

From the results, the MD method was identified as the most reliable and accurate for predicting \(\delta\) values for the excipients. Also, these results suggest that the COMPASS force-field is suitable for predicting the solubility of DTX in excipients. The MD method accounts for the interactions between atoms \(i.e.\) hydrogen-bonding) within a periodic boundary condition and the many conformations of the compounds. 171, 182 In contrast, GCM and the \(C^2\)-Synthia module only consider the contributions from the functional groups of a single molecule \(i.e.\) drug and excipients. 170 The advantages of GCM and \(C^2\)-Synthia module are that they allow for a fast and straightforward prediction of the \(\delta\) values.
2.4.3. **Evaluation of the degree of interaction within the binary mixed systems**

The plot of total energy as a function of time for the last 1100 ps demonstrated that the equilibrium stage was obtained for binary mixtures of DTX and excipients at $\phi_{\text{DTX}} = 0.025$ (Figure 2.3). No significant changes in the calculated cohesive energy of the binary mixed systems were observed over the last 200 ps of the simulation.\(^{187}\) Hence the calculated interaction parameter $\chi_{FH}$ were considered as equilibrium values at 25 °C.

![Figure 2.3: Nosé–Hoover constant-temperature, constant-volume ensemble equilibrium of docetaxel and (a) tributyrin, (b) tricaproin, (c) tricaprylin and (d) vitamin E pairs at volume fractions of docetaxel of 0.025 for the last 1100 ps of the simulation.](image)
The results from the MD simulations demonstrated that the $\chi_{FH}$ values for mixtures of DTX and excipient increased as a function of $\phi_{DTX}$ in the binary system (i.e. interactions between DTX and excipients decreased with increasing $\phi_{DTX}$). Consistent with the calculated $\delta_{HIL-MD}$ values and the experimental results, stronger interactions (i.e. smaller $\chi_{FH}$ values) were predicted for the DTX-tributyrin pair in comparison to DTX in the other excipients at the same $\phi_{DTX}$ (Figure 2.4). For $\phi_{DTX}$ ranging from 0.050 to 0.100, the values obtained for $\chi_{FH}$ can be related to the ability of the excipient to engage in hydrogen-bonding interactions with DTX. Specifically, similar $\chi_{FH}$ values were obtained for DTX in vitamin E ($\delta_h = 7.55$) and tricaproin ($\delta_h = 7.44$) at $\phi_{DTX}$ of 0.050; whereas, a lower $\chi_{FH}$ values was obtained for DTX in tributyrin ($\delta_h = 8.62$) in comparison to DTX in tricaprylin, vitamin E or tricaprylin ($\delta_h = 6.65$) as shown in Figure 2.4 and Table 2.4.

Figure 2.4: Flory-Huggins interaction parameter for the binary mixtures of docetaxel and tricaprylin (●), tricaproin (■), tributyrin (□), vitamin E (Δ), and β-caryophyllene (○) as a function of volume fraction of docetaxel.
For DTX and β-caryophyllene mixtures, the calculated $\chi_{FH}$ values obtained from the MD simulations were greater than 0.50 for all $\phi_{DTX}$ (0.025 – 0.100) indicating complete insolubility for DTX in these mixtures. These results were further supported by the poor solubility of DTX in β-caryophyllene (0.4mg/mL) that was observed experimentally (Table 2.3). Yet, a $\phi_{DTX}$ of 0.025 corresponds to a concentration of approximately 31 mg/mL of DTX in β-caryophyllene. In order to calculate the interaction between DTX and β-caryophyllene at lower $\phi_{DTX}$ (e.g. $\phi_{DTX} = 0.010$ or 12 mg/mL), a periodic cell containing one DTX and 290 β-caryophyllene molecules (e.g. 11,421 atoms) is required for MD simulation. However, MD simulation on a large periodic system (i.e. greater than 10,000 atoms) is impractical as it requires very long simulation times to reach the equilibrium stage. Therefore, simulations at low $\phi_{DTX}$ in β-caryophyllene were not performed.

In this study, the values for $\phi_{DTX}$ at $\chi_{FH} = 0.50$ obtained from Figure 2.4 were taken to be the maximum solubility of DTX in each excipient (Table 2.3). The values obtained for solubility via MD simulations for DTX in tributyrin, tricaproin or vitamin E were considered accurate in comparison to the experimental results as the percent deviation was between 2 and 6 %. However, the simulated solubility of DTX in tricaprylin (65 mg/mL) was approximately 15 % above the experimental value (56 mg/mL). The differences between the experimental and simulated solubility values may be attributed to several factors. Firstly, the value for the molar volume of DTX employed in the simulations was obtained using the C²-Synthia module and so considered to be equivalent to the molar volume in vacuo. Thus, the change in volume associated with mixing DTX and excipient was considered to be negligible. However, this likely only has a slight
affect on the accuracy of the results since $\phi_{\text{DTX}}$ in the binary mixtures is low. Specifically, the density of the amorphous cell for the binary mixtures of DTX and tributyrin ranged from 0.2 to 0.9% greater than the experimental density of pure tributyrin, whereas; the density for the binary mixtures of DTX and tricaprylin varied from 0.8 to 2.4% greater than pure tricaprylin. It should also be noted that the CED as well as the $\delta_{\text{HIL-MD}}$ were proportional to the volume of the amorphous cell as described in eq 2.9. Secondly, in theory, a homogeneous (i.e. one phase) solution of a two-component system is said to correspond to a value of less than 0.5 for $\chi _{\text{FH}}$. Yet, the assumption was made that the $\phi_{\text{DTX}}$ at $\chi_{\text{FH}} = 0.50$ could be converted to the maximum solubility for the compound in the excipient. In this way, the simulated values for solubility are expected to slightly overestimate the actual solubility of the drug in each excipient. Thirdly, as mentioned in the methods section, the MD simulations are based on a modified FH theory. This modified FH theory may overestimate the attractive intermolecular interactions that are present between DTX and the excipients. Thus, the simulated values for $\chi_{\text{FH}}$ are underestimated which results in an overestimate of the solubility of DTX in the excipients.

The experimental values obtained for the solubility of DTX in the various excipients were used to calculate the “critical” values for $\chi_{\text{FH}}$ (i.e. when phase separation begins to occur) using eq 2.13. The values for $\chi_{\text{FH, crit}}$ were found to be 0.54, 0.56, 0.57 and 0.59 for DTX in tricaprylin, vitamin E, tricaproin and tributyrin, respectively. These values are close to but slightly greater than the expected $\chi_{\text{FH, crit}}$ value of 0.5. This finding is in agreement with other studies that have shown experimentally
that the value of $\chi_{FH_{\text{crit}}}$ can vary depending on the nature and properties of the binary mixture. 45, 187, 188, 203-205

Overall, a good agreement was obtained between the experimental and simulated values for the solubility of DTX in the excipients. However, limitations of this MD simulation method were observed including the inability to estimate the solubility of the drug in poor excipients (i.e. $\beta$-caryophyllene). In addition, the discrepancy between the experimental and simulated values for solubility suggests that this method may be most reliably employed for relative ranking of excipients rather than determination of the absolute value of the solubility of a drug in a particular excipient.

2.5. Conclusion

Our computational model accurately predicted the relative solubility of DTX in the various excipients, as the computational results were in agreement with values obtained experimentally. Overall, of the excipients evaluated tributyrin was found to be the most favourable for solubilization of DTX. The GCM and the C$^2$-Synthia module were suitable for estimating $\delta$ values and screening excipients that are similar in structure (e.g. tributyrin, tricaproin and tricaprylin). The $\delta_{\text{HAN}}$ was useful for screening polar excipients that have the potential to engage in hydrogen-bonding interactions with DTX such as triglycerides and vitamin E. In order to estimate the solubility of DTX in excipients based on $\delta$ values, one also needs to consider both the $\Delta\delta$ values of the DTX-excipient pairs and the structures of the compounds. The MD simulation method using CED to calculate $\delta_{\text{HIL}}$ and $\chi_{FH}$ values was the optimal \textit{in silico} method for determining the solubility of DTX in excipients. The $\chi_{FH}$ values obtained from MD simulations were
shown to be useful for obtaining an accurate estimate of the solubility of DTX in excipients. Further studies will calculate the interaction energy associated with hydrogen-bonding between DTX and excipients. Overall, the present study demonstrated the validity of the computational model as a reliable analytical tool for designing drug formulations.

2.6. Acknowledgments

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Chapter 3: Enhancement of Docetaxel Solubility via Conjugation of Formulation-Compatible Moieties

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**Abstract:** Theoretical calculations were employed to direct the design of docetaxel conjugates with enhanced solubility in the internal phase of a nano-emulsion formulation. The theoretically-identified optimal docetaxel conjugates were synthesized by direct attachment of lauroyl moieties through an ester linkage to docetaxel. In comparison to docetaxel, the conjugates exhibited significantly improved solubility in the oil as predicted by our theoretical calculations. This contributed to high drug entrapment efficiencies (up to 97%) and a high drug loading capacity (5.7 % w/w) for the docetaxel conjugates. The mono-substitution of an acyl group at C-2’ of docetaxel resulted in a conjugate with thirty-seven- to forty-six-fold lower cytotoxicity than that of the parent drug in two human cancer cell lines. Importantly, the activity exerted by the mono-substituted docetaxel on the cancer cells was due in part to the cytotoxicity of the parent drug that was released via hydrolysis of the ester bond between the lauroyl moiety and the drug under biologically relevant conditions. In contrast, di- and tri-substitution of acyl groups at C-2’, C-7 and/or C-10 of docetaxel resulted in non-hydrolysable conjugates which were found to be inactive. Overall, our results show that theoretical calculation is a promising strategy for guiding the enhancement of material-drug compatibility in formulation development. As well, these studies confirm that chemical modification of docetaxel for enhancement of material-drug compatibility should be limited to mono-substitution at C-2’ and result in a prodrug that is hydrolysable at a moderate rate under biologically relevant conditions.
3.1. **Introduction**

It has been reported that about 40% of the small molecules that are emerging as new drug candidates are hydrophobic.\(^{206}\) Full exploitation of the therapeutic potential of hydrophobic drugs relies on their solubilization in a formulation or requires an advanced delivery system. However, low drug loading levels (i.e. low drug to material ratio) and poor drug retention in the delivery system following administration remain serious problems.\(^8,9\) In order to address these challenges, contemporary efforts are focused on optimizing the compatibility between the drug and the excipient (i.e. polymer or lipid) that forms the core of the delivery system.\(^{10,207-209}\) Optimization of the compatibility between the hydrophobic drug and core-forming material has been shown to result in significant improvements in formulation stability, drug loading capacity and drug retention.\(^{10}\)

Several strategies have been successfully utilized in order to enhance material-drug compatibility; of particular note are the following three approaches. First, one may pre-screen a library of materials against the drug of interest using semi-empirical and computational methods in order to identify the best material-drug pair.\(^{48,57,105,206}\) Second, chemical modification of the core-forming material of the delivery system has the potential to increase the suitability of the core as a microenvironment for solubilization of a specific drug (e.g. NK911 – a formulation of doxorubicin,\(^{209}\) NK105 – a formulation of paclitaxel (PTX)\(^{208}\)). Third, the drug molecules may be chemically modified or conjugated to generate a prodrug that has increased solubility, loading and/or retention in a specific formulation. For example, a study by Lundberg *et al.* demonstrated that the prodrug PTX-oleate is significantly more soluble in a cholesterol microemulsion formulation than PTX.\(^{10}\) As well, the prodrug PTX-docosahexanoic acid\(^{210}\) (i.e. Taxoprexin\(^\circledR\)) was found
to have a higher solubility than unmodified PTX in the conventional formulation of PTX that is known as Taxol® and formed from 10% Cremophor EL-P/10% ethanol/80% saline.\textsuperscript{210} Taxoprexin® has entered phase III clinical development, targeting patients with locally advanced or metastatic NSCLC.\textsuperscript{211}

Currently, the taxanes, namely PTX and docetaxel (DTX), are the most widely used hydrophobic drugs for the treatment of cancer.\textsuperscript{160, 161} Indeed PTX, in the commercially available formulation Taxol®, is approved for first line treatment of various cancers such as ovarian, breast, and non-small cell lung. The commercially available formulation of DTX, known as Taxotere® is approved for first-line treatment of prostate, gastric, lung, breast and head and neck cancers.\textsuperscript{127, 160, 161, 212} PTX and DTX exert their cytotoxic effect by binding β-tubulin and inhibiting microtubule depolymerisation, which leads to cell cycle arrest.\textsuperscript{161, 213} While PTX and DTX are structurally similar, and several pre-clinical and clinical studies have shown that DTX is at least as effective as PTX,\textsuperscript{160, 161, 214-218} their pharmaceutical characteristics have important distinctions. In comparison to PTX, DTX binds microtubules with greater affinity\textsuperscript{161} and \textit{in vitro} studies have revealed that DTX is more cytotoxic.\textsuperscript{213, 219} However, the commercially available formulation of DTX (i.e. Taxotere®)\textsuperscript{213} has side effects that are caused by the polysorbate 80 formulation vehicle, such as acute hypersensitivity reactions and vesicular degeneration.\textsuperscript{220-222} There is therefore an impetus to develop new formulations of DTX that are formed from safer excipients or materials.

With the intention of developing a safer delivery material to replace polysorbate 80, we recently generated nano-emulsion (NE)/nanocapsule formulations stabilized by a non-toxic poly(ethylene glycol) (PEG) derivative (i.e. Solutol HS® 15 \textsuperscript{223}) that was shown to
circulate for prolonged periods in vivo and accumulate preferentially at the tumor site.\textsuperscript{106, 109} The inner phase of this NE included FDA-approved medium chain triglycerides\textsuperscript{224} (i.e. caprylic/capric triglycerides; Labrafac\textsuperscript{TM}). However, the drug loading capacity of this NE formulation for DTX was not optimal; therefore large volumes of the NE formulation would need to be administered in order to achieve therapeutic drug levels. In order to address this deficiency, this study pursues the chemical modification of DTX in order to improve the compatibility between the drug and the inner phase of the NE formulation.

In another article, we described both molecular dynamics and semi-empirical methods for estimating the solubility of DTX in various pharmaceutical excipients and validated the methods experimentally.\textsuperscript{48} In this study, we employed a similar computational method to direct the chemical modification of DTX. The optimal DTX conjugates, identified by molecular simulation, were synthesized, characterized and evaluated in terms of solubility in Labrafac\textsuperscript{TM}. NE formulations of the DTX conjugates were then prepared and investigated in vitro in terms of drug entrapment efficiency and stability. The experimental studies on DTX conjugates demonstrate significant improvements in solubility, drug loading capacity and entrapment efficiency in the NE formulation. Evaluation of the in vitro cytotoxicity of the conjugates in SKOV-3 and H460 cancer cells revealed a marked decrease in the activity of DTX conjugates relative to DTX. Only the mono-substituted DTX conjugate was slowly hydrolyzed in vitro, cleaving the conjugated moiety from the parent drug and thus alleviating the conjugation-based inhibition of cytotoxicity, which is all but complete. Therefore, the hydroxyl group at C-2’ of DTX was identified as the optimal group for direct chemical modification of the drug to produce a conjugate with enhanced solubility in the formulation and capacity for regeneration of the biological activity of the
drug. Interestingly, the good agreement between the computational and the experimental solubility and hydrophobicity data suggests a bright future for theoretically-guided prodrug development of hydrophobic drugs.

3.2. Computational Methods

**Calculation of hydrophobicity and solubility parameters:** QSAR+ and Synthia, Cerius2, Accelrys Inc. (i.e. C2_QSAR+ and C2_Synthia)\(^ {225}\) were employed to estimate the octanol to water partition coefficient (log \(P_{o/w}\)) and solubility parameters of the compounds, respectively (Table 3.1). The log \(P_{o/w}\) model employed was established by Ghose and Crippen using the atom based approach in which each atom is assigned an additive value from a particular class.\(^ {226}\) The C2_Synthia module applies the Fedors approach to calculate the Hildebrand solubility parameters (\(\delta_{\text{HIL}}\)).\(^ {169}\) In a previous study, we examined the accuracy and reliability of various \textit{in silico} methods for use in the selection of a suitable excipient for solubilization of DTX.\(^ {48}\) In this study, the \(\delta_{\text{HIL}}\) values for DTX and a series of excipients were obtained using semi-empirical methods (i.e. group contribution method and \(C^2\)-Synthia module, Cerius\(^2\) software) and molecular dynamics simulation. Overall, the molecular dynamics simulation method produced the most accurate results but required significant time and computational resources. The semi-empirical method, relying on the \(C^2\)-Synthia module, was found to be fast, reliable and provided a good prediction of the relative degree of solubility of the drug in various structurally similar excipients. For these reasons the \(C^2\)-Synthia module was employed in the current research to obtain a rapid and straightforward prediction of the \(\delta_{\text{HIL}}\) and Log \(P_{o/w}\) values.
Theoretically, the smaller the difference between the values for $\delta_{\text{HIL}}$ of the solute and solvent, the greater the solubility of the solute in the solvent. In this study, the $\delta_{\text{HIL}}$ values were used to estimate the miscibility of the drugs (i.e. DTX conjugates and DTX) and Labrafac™ (i.e. tricaprylin and tricaprin) based on the chemical structures of the molecules. The log $P_{\text{o/w}}$ can be employed to predict the relative lipophilicity and retention of the drugs in the inner phase (i.e. Labrafac™) of the emulsion formulation.

3.3. Materials and Experimental

3.3.1. Materials

Anhydrous DTX (99.8%) was obtained from Sai Life Sciences (Hyderabad, India). Lauroyl chloride (98%) and 4-(dimethylamino)pyridine (DMAP) (99%) were purchased from Sigma Aldrich (Oakville, ON, Canada). High performance liquid chromatography (HPLC) grade solvents were obtained from Caledon (Georgetown, ON, Canada). Labrafac™ (i.e. Labrafac™ Lipo WL 1349) and Solutol® (i.e. Solutol® HS 15) were provided by Gattefossé Canada, Inc. (Toronto, ON, Canada) and BASF Corporation (Florham Park, NJ), respectively. Gibco® RPMI-1640 medium was acquired from Life Technologies (CA). All materials were used as received.

3.3.2. Synthesis and characterization of DTX conjugates

The conjugation of lauroyl chloride to DTX was monitored by thin layer chromatography (TLC) using chloroform:methanol:hexane (93:3:4, v/v/v) as solvent. The formation of the DTX-conjugates was verified using mass spectrometry (MS) and $^1$H NMR.
**Synthesis:** Briefly, DTX (500 mg, $6.19 \times 10^{-4}$ mol) and DMAP (151.2 mg, $12.38 \times 10^{-4}$ mol) were dissolved in 15 mL of dichloromethane in a 50 mL round-bottom flask. The reaction mixture was stirred for 10 minutes under nitrogen atmosphere at $0^\circ$C using an ice bath. Then, lauroyl chloride (147 μL, $6.19 \times 10^{-4}$ mol) was added dropwise to the mixture followed by stirring for six hours under nitrogen atmosphere at $0^\circ$C to produce the mono-substituted DTX conjugate, 2’-lauroyl-docetaxel ($\text{1L-DTX}$, retention factor ($R_f$) = 0.20). A second portion of DMAP (151.2 mg, $12.38 \times 10^{-4}$ mol) and lauroyl chloride (147 μL, $6.19 \times 10^{-4}$ mol) were added to the reaction mixture and stirred for another five hours under nitrogen atmosphere at $0^\circ$C. This yielded a mixture of two di-substituted DTX conjugates that were then separated by TLC to obtain the 2’,7-dilauroyl-docetaxel ($\text{2L}_{2’,7-}\text{DTX}$, $R_f = 0.70$) and 2’,10-dilauroyl-docetaxel ($\text{2L}_{2’,10-}\text{DTX}$, $R_f = 0.60$). Finally, a third portion of DMAP (151.2 mg, $12.38 \times 10^{-4}$ mol) and lauroyl chloride (147 μL, $6.19 \times 10^{-4}$ mol) were added to the reaction mixture and stirred for five hours under nitrogen atmosphere at room temperature to obtain 2’-7-10-trilauroyl-docetaxel ($\text{3L-DTX}$, $R_f = 0.90$).

**Purification:** The reaction mixture was diluted with diethyl ether, washed with 5% HCl and saline. The solution of DTX conjugate was collected, dried and dissolved in acetonitrile. This solution was then stored at $0^\circ$C overnight followed by filtration to further remove the precipitated impurities. The collected solution was concentrated and applied to a silica gel column with chloroform:methanol:hexane (20 mL of 95:1:4, 20 mL of 94:2:4 and 40 mL of 93:3:4, v/v/v) as mobile phase to obtain the pure DTX conjugates. The yields for the conjugation reactions were greater than 70% in all cases.
3.3.3. Analytical measurements

**Nuclear magnetic resonance (NMR):** The $^1$H NMR spectra were measured with a Mercury 300 spectrometer at 300 MHz in CDCl$_3$. The $^1$H chemical shifts are reported in Table 3.2 relative to the internal standard trimethylsilane (TMS) ($\delta = 0.00$ ppm).

**Mass spectroscopy:** Electrospray Ionization (ESI) was employed on an AB/Sciex QStar mass spectrometer (Applied Biosystems, Foster City, CA) connected to an Agilent 1100 capillary LC system (Agilent Technologies Canada Inc. Mississauga, ON) to determine the chemical composition of the DTX conjugates.

**High performance liquid chromatography (HPLC) analysis:** The concentration of DTX conjugate and DTX was measured using an HPLC (Agilent series 1200 Liquid Chromatograph, Agilent Technologies Canada Inc. Mississauga, ON) equipped with a Waters UV/VIS detector (Waters Inc., Milford, MA), Agilent sample processor and a Waters X Terra C$_{18}$ reverse-phase column (particle size, 5 µm) of dimensions 4.6 x 250 mm. DTX conjugates and DTX were detected at a wavelength of 227 nm. The flow rate was 1.0 mL/min. The mobile phase used for analysis of DTX (retention time ($R_T$) = 8 min) was acetonitrile and water (53:47, v/v). Mixtures of 2-propanol, acetonitrile and water (40:45:15, v/v/v) were used to isocratically elute 1L-DTX ($R_T = 5.1$ min), 2L$_{2',7'}$-DTX ($R_T = 22.4$ min) and 2L$_{2',10'}$-DTX ($R_T = 20.5$ min). The 3L-DTX was eluted isocratically at 7.0 min and greater than 60 min using 2-propanol:acetonitrile:water with a volume ratio of 74:20:6 and 40:45:15 as mobile phase, respectively.

**Dynamic light scattering (DLS):** DLS was used to determine the size and distribution of the emulsion at an angle of 90° at room temperature (90Plus Particle Size
3.3.4. Evaluation of relative solubility

The relative solubility of DTX and conjugates (i.e. 1L-DTX, 2L-DTX and 3L-DTX) were measured in Labrafac™ at room temperature. Briefly, known amounts of DTX or DTX conjugate were added to Labrafac™ and the mixture was vortexed and stirred at room temperature for 24 hours. The samples were then centrifuged in Eppendorf tubes for 1 hour at 20 000 × g (Eppendorf 5804R, Eppendorf Inc., Hamburg, Germany) to remove undissolved drug. Following centrifugation, the saturated solution (i.e. the supernatant) was separated and analyzed by HPLC. The saturation of the solution containing DTX was confirmed by the presence of precipitate on the bottom of the Eppendorf tube. However, precipitate was not observed in the Labrafac™ solutions containing high concentrations of DTX conjugates. The relative solubility of the conjugate in this study, determined by using HPLC, was defined as the concentration of the DTX conjugate in the Labrafac™ unsaturated solution.

3.3.5. Preparation and characterization of the nano-emulsion

Formulations containing low (i.e. 0.6 % and 1.2 % w/w) and high drug concentrations (i.e. 5.7 % w/w) were prepared and investigated in terms of stability, drug loading and drug entrapment efficiency. The compositions of the nano-emulsions (NEs) are provided in Table 3.4. The NEs were prepared using a slightly modified procedure that was reported in the literature. Briefly, drug (i.e. DTX conjugate or DTX) was measured after 1:400 dilutions using filtered double distilled water.
dissolved and stirred in Labrafac™ at room temperature for 24 h followed by stirring at 60°C for 10 min. To this solution, Solutol® was added and stirred (630 rpm) for 15 min at 40°C. The dispersing phase (0.9% w/v NaCl) was then added to this mixture with agitation (840 rpm) and stirred for another 10 min under the same conditions. Finally, the formulation was sonicated for 3 h using a Bransonic 1510 (Branson, Danbury, CT). The mean hydrodynamic diameter and size distribution were determined in triplicate by DLS (Table 3.4). HPLC analysis was used to confirm that the drug and conjugates remained intact following NE preparation. The physical stability of the NE was assessed by size measurements. The NEs were observed under a VWR VistaVision microscope (Mississauga, Ontario, Canada) in order to verify if crystallization of DTX or DTX conjugates had occurred.

### 3.3.6. Determination of drug loading and entrapment efficiency

The entrapment efficiency (EE) of the NE formulations of DTX or DTX conjugates were measured using both ultracentrifugation and dialysis methods in triplicate.\(^{227}\)

**Ultracentrifugation:** Briefly, a 1.0 mL aliquot of an NE sample was placed in the outer chamber of an ultra-filter tube (molecular weight cut-off 10 kDa; Millipore Co., Bedford, USA) and centrifuged at 2500 \(\times\) g for 1 hour at room temperature. The dispersion medium along with the free drug was separated from the NE sample through the ultrafiltration device. The concentration of drug in the dispersion medium (inner chamber) and the supernatant (outer chamber) were measured using HPLC.
**Dialysis**: The free drug in the dispersion medium was removed using the dialysis method.\(^{227}\) Briefly, 200 μL of NE sample was added to dialysis tubing (molecular weight cutoff 10 kDa) and suspended in saline (1 L) at room temperature for 40 min. The concentration of drug remaining in the dialysis tubing was measured by HPLC analysis. Entrapment efficiency was calculated using the following equation:

\[
EE(\%) = \frac{\text{Weight of drug in the supernatant or dialysis bag}}{\text{Initial weight of drug loaded}} \times 100
\]  \hspace{1cm} (3.2)

### 3.3.7. Evaluation of \textit{in vitro} cytotoxicity and hydrolysis

The cytotoxicity of DTX and DTX conjugates were evaluated in triplicate in SKOV-3 human ovarian and H460 human non-small cell lung cancer cells using RPMI 1640 as cell growth media, as described elsewhere.\(^{228}\) Briefly, cells were seeded in 96 well plates with a cell density of 5,000 cells/well followed by a 24 hour incubation period. The cell growth media was then replaced with 150 μL of fresh media containing the appropriate amounts of unmodified DTX, \(1L\text{-DTX}, 2L_{2',7}\text{-DTX}\) and \(3L\text{-DTX}\) \((n = 3)\). Following 72 hours incubation, the MTT assay was employed to determine the cell viability by measuring optical absorbance at 570 nm using a Spectra Max plus microplate reader (Molecular Devices, Sunnyvale, CA). The cells incubated with drug free media (i.e. the control) were considered to be 100 % viable.

The hydrolysis of DTX conjugates was studied in RPMI media in triplicate. Briefly, solutions of DTX conjugates \((350 \mu M \text{ DTX equivalent})\) in RPMI were incubated at 37°C for 72 hours. The concentration of DTX, released via hydrolysis of the ester bond between the lauroyl moiety and the drug, was measured by HPLC analysis.
3.4. Results and Discussion

3.4.1. Theoretical hydrophobicity and solubility parameters

Lipidic nanoformulations that contained Labrafac™ as the core component have been shown to be stable in vivo with a prolonged circulation time and significant accumulation in solid tumors. However, administration of large volumes of this emulsion would be required in order to achieve therapeutic drug levels due to the limited drug loading capacity of the NE formulation for DTX. Replacement of the inner phase of the NE with other excipients resulted in either less drug loading (i.e. poor solubility) or serious toxic side-effects. In light of the fact that the extent of drug loading and retention can be significantly improved by increasing the compatibility between the drug and the core of the delivery system, we set out to investigate the conjugation of DTX to saturated fatty acid moieties that are structurally similar to Labrafac™ in terms of theoretical Log P<sub>o/w</sub> and δ<sub>HIL</sub> values.

The Log P<sub>o/w</sub> and δ<sub>HIL</sub> values of DTX, DTX conjugates and Labrafac™ were determined by molecular simulation (Table 3.1). The δ<sub>HIL</sub> of Labrafac™ (i.e. mainly a mixture of tricaprin and tricaprylin) is an average of the δ<sub>HIL</sub> of tricaprylin (δ = 17.30 (J/cm<sup>3</sup>)<sup>1/2</sup>) and tricaprin (δ<sub>HIL</sub> = 17.80 (J/cm<sup>3</sup>)<sup>1/2</sup>). For each drug-Labrafac™ pair, the difference between the δ<sub>HIL</sub> of drug and δ<sub>HIL</sub> of Labrafac™ (Δδ<sub>HIL</sub>) was calculated in order to estimate their relative miscibility. A lower value of Δδ<sub>HIL</sub> for a specific drug-Labrafac™ pair is predicted to result in a higher solubility for the drug in Labrafac™. A higher value for the calculated Log P<sub>o/w</sub> is indicative of a more lipophilic drug and greater degree of compatibility between the drug and the oil phase. Importantly, based on the Log P<sub>o/w</sub> values for a total of ten compounds (Table 3.1), a linear relationship between the
calculated Log $P_{o/w}$ values and the total number of carbon atoms in the acyl chains conjugated to DTX was obtained ($R^2 = 0.997$). The relationship was:

$$\text{Log } P_{o/w} = 0.44 \times (\text{no. carbon atoms in all acyl chains conjugated to DTX}) + \text{Log } P_{o/w} \text{ (DTX)}$$

(3.1)

Table 3.1: Docetaxel and candidate docetaxel conjugates. The octanol to water partition coefficients (Log $P_{o/w}$) and solubility parameters ($\delta_{\text{HIL}}$) were calculated using QSAR+ and C2.Synthia, respectively.

<table>
<thead>
<tr>
<th>Molecular Weight (g/mol)</th>
<th>C10:0 (x = 8)</th>
<th>C12:0 (x = 10)</th>
<th>C14:0 (x = 12)</th>
<th>C10:0 (x = 8)</th>
<th>C12:0 (x = 10)</th>
<th>C14:0 (x = 12)</th>
<th>C10:0 (x = 8)</th>
<th>C12:0 (x = 10)</th>
<th>C14:0 (x = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Docetaxel</strong></td>
<td>807.9</td>
<td>962.1</td>
<td><strong>990.2</strong></td>
<td>1018.2</td>
<td>1144.4</td>
<td>1172.5</td>
<td>1228.6</td>
<td>1298.7</td>
<td>1354.8</td>
</tr>
<tr>
<td><strong>Log $P_{o/w}$</strong></td>
<td>2.45</td>
<td>6.69</td>
<td>7.60</td>
<td>8.51</td>
<td>11.84</td>
<td>12.75</td>
<td>14.57</td>
<td>16.08</td>
<td>17.90</td>
</tr>
<tr>
<td><strong>$\delta_{\text{HIL}}$ (J/cm$^3$)$^{1/2}$</strong></td>
<td>24.22</td>
<td>22.66</td>
<td><strong>22.49</strong></td>
<td>22.33</td>
<td>21.44</td>
<td><strong>21.33</strong></td>
<td>21.13</td>
<td>20.65</td>
<td><strong>20.50</strong></td>
</tr>
<tr>
<td><strong>$\Delta\delta_{\text{HIL}}$</strong></td>
<td>6.67</td>
<td>5.11</td>
<td><strong>4.94</strong></td>
<td>4.78</td>
<td>3.89</td>
<td><strong>3.78</strong></td>
<td>3.58</td>
<td>3.1</td>
<td><strong>2.95</strong></td>
</tr>
</tbody>
</table>

(a) $\Delta\delta_{\text{HIL}} = |\delta_{\text{HIL, drug}} - \delta_{\text{HIL, Labrafac}}|$; $\delta_{\text{HIL}}$ of Labrafac™ is 17.55 which is an average of $\delta_{\text{HIL}}$ values for tricaprylin ($\delta_{\text{HIL}} = 17.30$) and tricaprin ($\delta_{\text{HIL}} = 17.80$)
According to this relationship, each carbon atom in the attached acyl chain contributes approximately $0.44 \pm 0.01$ to the log $P_{o/w}$ value for the DTX conjugate. As shown in Table 3.1, lower $\Delta \delta_{HIL}$ and greater Log $P_{o/w}$ values were obtained for DTX conjugated with longer hydrocarbon chains and/or more lipophilic side chains per drug molecule. Studies have shown that the toxicity of fatty acids in Jurkat (T-lymphocyte) and Raji (B-lymphocyte) human cell lines decreased when the hydrocarbon chain length was decreased. The tolerable concentration of lauric acid (C12:0) in human cell lines was quite high (i.e. 200 $\mu$M) in comparison to the cytotoxicity of DTX in a range of human tumor cell lines (0.13 nM to 24 nM). Furthermore, Immordino et al. reported that the interaction of a lipophilic prodrug with a liposome bilayer increased when the acyl chain of the prodrug penetrated into the lipid bilayer and completely overlapped with the hydrophobic tails of phospholipids, thus improving the stability of the liposome. For these reasons, DTX conjugates with laurate moieties (C12:0, Table 3.1) were synthesized in order to produce conjugates with relatively non-toxic acyl chains that allow for full overlap with Labrafac$^{TM}$ (i.e. capric/caprylic glycerides), and yet have relatively moderate molecular weights and Log $P_{o/w}$ values.

3.4.2. Characterization of DTX conjugates

In this study, DTX was mono, di and trisubstituted with laurate moieties using a direct and efficient method that produced the DTX conjugates in high yield. The lauroyl chloric acid group of the fatty acid was directly conjugated to the hydroxyl (OH) group of DTX in a single step. Site-specific conjugation of fatty acid moieties to the OH groups of DTX was achieved by variation of the temperature during the reaction procedure.
The conjugation of the fatty acid moieties to DTX was confirmed by $^1$H-NMR (Figure 3.1). The $^1$H NMR chemical shifts for the conjugated fatty acid moieties on DTX conjugates are recorded in Table 3.2. The proton intensities are in accordance with the structure of the drugs. The $^1$H shifts for DTX and DTX conjugates were comparable to those reported previously for DTX and DTX derivatives. The $^1$H proton chemical shifts for the hydroxyl hydrogens at the C-2' (HO-C-2', 3.35 ppm), C-7 (HO-C-7, 1.50 ppm) and C-10 (HO-C-10, 4.20 ppm) atoms on DTX disappeared following conjugation of the fatty acid moieties. Furthermore, the $^1$H chemical shift for the hydrogens connected to C-2' (HC-2', 4.62 ppm), C-7 (HC-7, 4.24 ppm) and C-10 (HC-10, 5.20 ppm) atoms resonated at lower field strengths due to the conjugation.
Figure 3.1. $^1$H NMR spectra of (a) 1L-DTX, (b) 2L$_{2',7}$-DTX (c) 2L$_{2',10}$-DTX and (d) 3L-DTX
Table 3.2: $^1$H NMR chemical shifts for docetaxel, 1L-DTX, 2L$_2$'7-DTX, 2L$_2$'10-DTX and 3L-DTX

<table>
<thead>
<tr>
<th>Position</th>
<th>1H</th>
<th>1L-DTX</th>
<th>2L$_2$-10-DTX</th>
<th>2L$_2$-7-DTX</th>
<th>3L-DTX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ (ppm) (Hz)</td>
<td>δ (ppm) (Hz)</td>
<td>δ (ppm) (Hz)</td>
<td>δ (ppm) (Hz)</td>
<td>δ (ppm) (Hz)</td>
</tr>
<tr>
<td>2</td>
<td>1H</td>
<td>5.68 (d, 7.1)</td>
<td>5.69 (d, 7.2)</td>
<td>5.69 (d, 7.1)</td>
<td>5.69 (d, 7.0)</td>
</tr>
<tr>
<td>3</td>
<td>1H</td>
<td>3.91(d, 7.0)</td>
<td>3.94 (d, 7.3)</td>
<td>4.02 (d, 7.1)</td>
<td>4.02 (d, 7.0)</td>
</tr>
<tr>
<td>5</td>
<td>1H</td>
<td>4.94(dd, 9.7, 2.1)</td>
<td>4.96(dd, 9.6, 2.2)</td>
<td>4.95(dd, 9.7, 2.2)</td>
<td>4.95(dd, 9.7, 2.2)</td>
</tr>
<tr>
<td>6</td>
<td>Ha</td>
<td>2.59(dt, 3.2, 7.2)</td>
<td>2.59(dt, 3.3, 7.4)</td>
<td>2.53(ddd, 4.7, 9.2, 17.2)</td>
<td>2.62(dt, 2.7, 7.3)</td>
</tr>
<tr>
<td>6</td>
<td>Hb</td>
<td>1.85 (m)</td>
<td>1.85 (m)</td>
<td>1.92 (m)</td>
<td>1.92 (m)</td>
</tr>
<tr>
<td>7</td>
<td>1H</td>
<td>4.24 (m)</td>
<td>4.26 (m)</td>
<td>4.19 (m)</td>
<td>5.59 (m)</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>1.57 (br)</td>
<td>1.53 (br)</td>
<td>1.54 (br)</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>1H</td>
<td>5.20 (d, 1.6)</td>
<td>5.21 (d, 1.4)</td>
<td>6.31 (s)</td>
<td>5.34 (d, 1.20)</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>4.20 (d, 1.7)</td>
<td>4.18 (d, 1.4)</td>
<td>-</td>
<td>3.94 (d, 1.6)</td>
</tr>
<tr>
<td>13</td>
<td>1H</td>
<td>6.22 (t, 8.6)</td>
<td>-</td>
<td>6.24 (t, 8.8)</td>
<td>6.24 (t, 8.8)</td>
</tr>
<tr>
<td>14</td>
<td>Ha</td>
<td>2.27 (d, 9.2)</td>
<td>2.34 (d, 8.2)</td>
<td>2.35 (d, 8.6)</td>
<td>2.35 (d, 8.7)</td>
</tr>
<tr>
<td>16</td>
<td>3H</td>
<td>1.24 (s)</td>
<td>1.22 (s)</td>
<td>1.22 (s)</td>
<td>1.22 (s)</td>
</tr>
<tr>
<td>17</td>
<td>3H</td>
<td>1.13 (s)</td>
<td>1.12 (s)</td>
<td>1.09 (s)</td>
<td>1.09 (s)</td>
</tr>
<tr>
<td>18</td>
<td>3H</td>
<td>1.85 (s)</td>
<td>1.96 (s)</td>
<td>2.00 (s)</td>
<td>2.00 (s)</td>
</tr>
<tr>
<td>19</td>
<td>3H</td>
<td>1.76 (s)</td>
<td>1.75 (s)</td>
<td>1.84 (s)</td>
<td>1.84 (s)</td>
</tr>
<tr>
<td>20</td>
<td>Ha</td>
<td>4.32(d, 8.5)</td>
<td>4.33 (d, 8.4)</td>
<td>4.34 (d, 8.4)</td>
<td>4.34 (d, 8.4)</td>
</tr>
<tr>
<td></td>
<td>Hb</td>
<td>4.19 (d, 8.5)</td>
<td>4.20 (d, 8.4)</td>
<td>4.21 (d, 8.6)</td>
<td>4.21 (d, 8.7)</td>
</tr>
<tr>
<td>22</td>
<td>3H</td>
<td>2.37 (s)</td>
<td>2.44 (s)</td>
<td>2.44 (s)</td>
<td>2.44 (s)</td>
</tr>
<tr>
<td>25 and 29</td>
<td>2H</td>
<td>8.11 (d, 7.3)</td>
<td>8.12 (d, 7.1)</td>
<td>8.12 (d, 7.1)</td>
<td>8.12 (d, 7.1)</td>
</tr>
<tr>
<td>26 and 28</td>
<td>2H</td>
<td>7.50 (t, 7.3)</td>
<td>7.50 (t, 7.2)</td>
<td>7.50 (t, 7.3)</td>
<td>7.50 (t, 7.2)</td>
</tr>
<tr>
<td>27</td>
<td>1H</td>
<td>7.61 (t, 7.5)</td>
<td>7.61 (t, 7.2)</td>
<td>7.61 (t, 7.4)</td>
<td>7.61 (t, 7.4)</td>
</tr>
<tr>
<td>31 and 35</td>
<td>2H</td>
<td>7.35-7.42 (m)</td>
<td>7.34-7.43 (m)</td>
<td>7.35-7.43 (m)</td>
<td>7.35-7.42 (m)</td>
</tr>
<tr>
<td>32 and 34</td>
<td>4H</td>
<td>7.35-7.42 (m)</td>
<td>7.34-7.43 (m)</td>
<td>7.35-7.43 (m)</td>
<td>7.35-7.42 (m)</td>
</tr>
<tr>
<td>33</td>
<td>1H</td>
<td>7.32 (m)</td>
<td>7.30 (m)</td>
<td>7.30 (m)</td>
<td>7.30 (m)</td>
</tr>
<tr>
<td>2'</td>
<td>1H</td>
<td>4.62 (br)</td>
<td>5.44 (br)</td>
<td>5.44 (br)</td>
<td>5.44 (br)</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>3.34 (d, 5.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>1H</td>
<td>5.26 (d, 8.6)</td>
<td>5.36 (d, 9.5)</td>
<td>5.34 (d, 9.6)</td>
<td>5.35 (d, 7.4)</td>
</tr>
<tr>
<td>4'</td>
<td>1H</td>
<td>5.42 (d, 9.4)</td>
<td>5.37 (d, 2.9)</td>
<td>5.39 (d, 2.8)</td>
<td>5.39 (d, 3.1)</td>
</tr>
<tr>
<td>7', 8', 9'</td>
<td>9H</td>
<td>1.34 (s)</td>
<td>1.33 (s)</td>
<td>1.34 (s)</td>
<td>1.34 (s)</td>
</tr>
</tbody>
</table>

Laurate side chain(s)

<table>
<thead>
<tr>
<th>Chain</th>
<th># L × 3H</th>
<th># L × 8 x 2H</th>
<th># L × 2H</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>0.88 (m)</td>
<td>0.88 (m)</td>
<td>0.88 (m)</td>
</tr>
<tr>
<td>(CH$_2$)$_8$</td>
<td>1.25 (m)</td>
<td>1.27 (m)</td>
<td>1.24 (m)</td>
</tr>
<tr>
<td>C$_\beta$ at C-2'</td>
<td>2H</td>
<td>2.34 (m)</td>
<td>2.35 (m)</td>
</tr>
<tr>
<td>C$_\beta$ at C-7</td>
<td>2H</td>
<td>2.22 (t, 7.3)</td>
<td>2.22 (t, 7.5)</td>
</tr>
<tr>
<td>C$_\beta$ at C-10</td>
<td>2H</td>
<td>2.22 (m)</td>
<td>2.22 (m)</td>
</tr>
<tr>
<td>C$_\alpha$</td>
<td># L × 2H</td>
<td>1.53 (m)</td>
<td>1.59 (m)</td>
</tr>
</tbody>
</table>

s: singlet; d: doublet; dd: doublet of doublet; t: triplet, dt: doublet of a triplet; m: multiplet; br: broad (a) refers to the structural formula given in Table 3.1 (b) chemical shift, multiplicity and coupling constant (J), (c) Total number of H = number of laurate side chains × number of hydrocarbon groups) × 2H.
For example, as shown in Figure 3.1b, the $^1$H-NMR for $1\text{L-DTX}$ verified the conjugation of lauroyl chloride to the OH group at the C-2' atom, via the absence of the peak at 3.35 ppm (HO-C-2') and a shift in the peak at 4.62 ppm (br) to 5.45 ppm (br). The calculated mass of $1\text{L-DTX}$ (989.5) agreed with the data obtained via ESI analysis, (990.5 [M + H$^+$], 1007.5 [M + NH$_4^+$] and 1012.5 [M + Na$^+$]).

As demonstrated in the $^1$H-NMR for $2\text{L}_{2',7}\text{-DTX}$ (Figure 3.1c), in addition to the observations in the $^1$H-NMR for $1\text{L-DTX}$, the peak at 1.50 ppm (HO-C-7, br) was not detected whereas the peak at 4.24 ppm (m, HC-7) was reallocated to 5.59 ppm (q) verifying the formation of $2\text{L}_{2',7}\text{-DTX}$. The formation of $2\text{L}_{2',10}\text{-DTX}$ was demonstrated by $^1$H-NMR based on the disappearance of the peak at 4.20 ppm (d, HO-C-10) and a shift in the peak corresponding to the hydrogen at the C-10 position from 5.20 ppm (d) to 6.31 ppm (d). The mass spectra of $2\text{L-DTX}$ obtained by ESI analysis showed a protonated molecule [M + H$^+$] at 1172.7 that agreed with the calculated mass (1171.7).

The above NMR observations for $1\text{L-DTX}$, $2\text{L}_{2',7}\text{-DTX}$ and $2\text{L}_{2',10}\text{-DTX}$ were also found for $3\text{L-DTX}$ confirming the formation of $3\text{L-DTX}$. The calculated mass of $3\text{L-DTX}$ was 1353.9, which agrees with the mass obtained by ESI, i.e. 1371.9 [M + NH$_4^+$].

Previous studies on the conjugation of PTX to hyaluronic acid have revealed that hyaluronic acid is preferentially conjugated to the OH-C-2’ group in PTX, rather than the more sterically hindered OH-C-7 group.$^{233-235}$ Therefore, it was expected that the conjugation of DTX to fatty acid moieties would preferentially occur at the OH-C2’ group of DTX. Amongst secondary conjugation sites, the OH-C-7 group is predicted to have slightly higher chemical reactivity toward the conjugation of a fatty acid than the OH-C-10 group. In fact, the formation of $2\text{L}_{2',7}\text{-DTX}$ product was found to be
approximately 4.5 times greater than $2L_{2',10}$-DTX. These results show that the OH-C-2’ group is the most favourable conjugation site for fatty acid moieties, followed by the OH-C-7 group and, finally, the more sterically hindered OH-C-10 group.

### 3.4.3. Experimental lipophilicity and solubility

In order to validate the results obtained by molecular simulation, the relative lipophilicity of DTX and conjugates were determined based on their retention time obtained from the HPLC chromatograms and compared to the theoretically calculated Log $P_{o/w}$ values. It is worth noting that a C$_{18}$ reverse-phase column was used for HPLC analysis, hence under similar conditions less lipophilic molecules are eluted earlier than more lipophilic molecules. According to the retention time of DTX and conjugates obtained from HPLC using 2-propanol:acetonitrile:water (40:45:15, v/v/v) as mobile phase, DTX ($R_T = 2.8$ min) was the least lipophilic molecule followed by $1L$-DTX ($R_T = 5.1$ min), $2L_{2',10}$-DTX ($R_T = 20.5$ min), $2L_{2',7}$-DTX ($R_T = 22.4$ min) and then $3L$-DTX ($R_T > 60$ min). Similarly, the theoretically calculated Log $P_{o/w}$ values increased with number of conjugated acyl chains. Therefore, the relative lipophilicity of each of the conjugates was found to be in good agreement with the Log $P_{o/w}$ values and may be tuneable by varying the acyl chain length and the number of side chains attached to DTX (eq.1).

The experimental solubility of DTX and DTX conjugates were determined and employed to validate the results obtained by molecular simulation. In comparison to the solubility of DTX in Labrafac™, only the relative solubilities of the DTX conjugates were determined due to their high solubility in Labrafac™ (27 % w/w of $1L$-DTX, 34 %
w/w of 2L-DTX or 3L-DTX in the Labrafac™ unsaturated solution). As listed in Table 3.3, the relative solubility of the DTX conjugates in Labrafac™ (> 345 mg/mL DTX equivalent) is more than eight-fold higher than the solubility of unmodified DTX in Labrafac™ (43.7 mg/mL or 4% w/w of DTX in Labrafac™ saturated solution). It is well known that the solubility of a compound is based on the concept of “like dissolves like”. In comparison to unmodified DTX, DTX conjugates (i.e. 1L-DTX, 2L-DTX, 3L-DTX) are more lipophilic as indicated by their higher LogP_o/w values and longer retention times observed by HPLC analysis. Therefore, the DTX-conjugates have a greater solubility in Labrafac™. In addition, the significant enhancement in the miscibility of the DTX conjugates is attributed to the similarity of the chemical structure of Labrafac™ and the laurate moieties that are conjugated to DTX (Table 3.1). In comparison to the previously reported solubility for DTX in various triglycerides, the solubility of DTX conjugates in Labrafac™ is remarkably greater than the solubility of DTX in tributyrin (108 mg/mL) and Labrafac™ (43.7 mg/mL).

**Table 3.3.** Solubility and cytotoxicity of docetaxel (DTX) and DTX conjugates

<table>
<thead>
<tr>
<th>DTX or DTX Conjugates</th>
<th>Solubility * (mg/mL)</th>
<th>IC_{50}^b (nM) SKOV-3</th>
<th>IC_{50}^b (nM) H460</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTX</td>
<td>43.7 ± 0.5</td>
<td>1.8 ± 0.2</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>1L-DTX</td>
<td>&gt; 368 (300)</td>
<td>82 ± 16</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>2L_{2',7}-DTX</td>
<td>&gt; 500 (345)</td>
<td>&gt; 2 \times 10^5</td>
<td>&gt; 2 \times 10^5</td>
</tr>
<tr>
<td>3L-DTX</td>
<td>&gt; 500 (298)</td>
<td>&gt; 2 \times 10^5</td>
<td>&gt; 2 \times 10^5</td>
</tr>
</tbody>
</table>

(a) Values in parentheses are equivalent concentration of DTX. (b) Data are presented as mean of DTX equivalent ± SD (n=3). The chemical structure of 1L-DTX (mono-substituted), 2L_{2',7}-DTX (di-substituted) and 3L-DTX (tri-substituted) are shown in Table 3.1 with x = 10.
3.4.4. Stability, drug loading and entrapment efficiency

NE formulations, outlined in Table 3.4, were prepared with low (i.e. formulation A) and high (i.e. formulation B) initial amounts of DTX or DTX equivalent. The amount of DTX or DTX equivalent initially added in DTX-A (formulation of DTX), 1L-DTX-A (formulation of 1L-DTX) and 2L-DTX-A (formulation of 2L2',7-DTX) formulations was 0.6 % by weight of the NE (i.e. 100% × initial weight of DTX or DTX equivalent ÷ the total mixture of Solutol® and Labrafac™). The DTX-B formulations were prepared initially with 1.2 % by weight of DTX per mL of the NE, whereas the 1L-DTX-B and 2L-DTX-B formulations were initially prepared with 5.7 % by weight of DTX equivalent per mL of the NE.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>DTX or DTX equiv. added a mg</th>
<th>DTX or DTX equiv. loaded b % w/w</th>
<th>Drug EE by ultracentrifuge %</th>
<th>Drug EE by dialysis %</th>
<th>Diameter nm</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE-control</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>198 ± 3</td>
<td>0.16 ± 0.20</td>
</tr>
<tr>
<td>DTX-A</td>
<td>15.0 (13.4)</td>
<td>0.6 (0.5)</td>
<td>90 ± 3</td>
<td>81 ± 6</td>
<td>199 ± 12</td>
<td>0.68 ± 0.88</td>
</tr>
<tr>
<td>DTX-B</td>
<td>30.0 (2.9)</td>
<td>1.2 (0.1)</td>
<td>10 ± 1</td>
<td>7 ± 1</td>
<td>182 ± 26</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>1L-DTX-A</td>
<td>15.0 (13.8)</td>
<td>0.6 (0.6)</td>
<td>92 ± 4</td>
<td>80 ± 4</td>
<td>203 ± 7</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>1L-DTX-B</td>
<td>150.0 (133.4)</td>
<td>5.7 (5.1)</td>
<td>89 ± 6</td>
<td>78 ± 3</td>
<td>184 ± 9</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>2L-DTX-A</td>
<td>15.0 (14.6)</td>
<td>0.6 (0.6)</td>
<td>97 ± 1</td>
<td>85 ± 5</td>
<td>167 ± 9</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>2L-DTX-B</td>
<td>150.0 (137.1)</td>
<td>5.7 (5.2)</td>
<td>91 ± 1</td>
<td>85 ± 3</td>
<td>206 ± 5</td>
<td>0.18 ± 0.03</td>
</tr>
</tbody>
</table>

Nanoemulsion (NE) samples were prepared in triplicate. Constant amounts of Labrafac™ (1485 mg, 2.9 mmol), Solutol® (990 mg, 1 mmol) and NaCl 0.9 (w/v, 12.46 mL) were added to each NE formulation. (a) The amount of docetaxel (DTX) or DTX equivalent initially added and actually loaded (values in parentheses). (b) The percent of DTX equivalent initially added and actually loaded (values in parentheses) was determined as the weight percentage of DTX equivalent relative to the total weight of NE (drug, Labrafac™ and Solutol®) excluding the dispersion media. (d) Drug entrapment efficiency (EE) was calculated as the percentage of drug actually loaded relative to total drug initially added.
The stability of the NE formulations was observed via microscopic analysis. As discussed previously, the amount of drug loaded in the NE formulations depends on the solubility or miscibility of the drug in Labrafac™, which is the internal phase of the NE. Super-saturation of the drug in the NE formulations can lead to drug crystallization or precipitation, which was observed by microscopic analysis for DTX-B formulation. Drug loaded NEs were in the range of 167 to 206 nm in diameter and stable over 24 hrs of storage at room temperature as determined by DLS. Following 48 hours of storage at room temperature, DLS analysis of the NEs revealed bimodal population distributions with diameters of 180 – 250 nm and 300 – 500 nm for each population.

The drug loading and EEs were determined using the ultracentrifugation and dialysis methods that have been validated as efficient methods for measuring drug EE in parenteral emulsions, liposomes and micelles formulations. The molecular weight cutoff of the dialysis membrane (10 kDa) only allows for diffusion of the free DTX or DTX conjugates. Following dialysis, the drug entrapped NE in the dialysis bag was collected and analyzed by HPLC. In comparison, the ultracentrifugation method allows non-entrapped drug and the dispersion medium from the outer chamber to pass through a filter (molecular weight cutoff 10 kDa). Following ultracentrifugation, the drug entrapped NE in the supernatant and the dispersion medium were collected in separate chambers and analyzed by HPLC. In comparison to the drug EE obtained with the ultracentrifugation method, the EE measured using the dialysis method was 3 % to 12 % lower. The lower drug EE obtained from the dialysis method can be attributed to additional loss of entrapped drug that occurs following loss of the free drug and the equilibration process.
About 90% of DTX was entrapped in the emulsion when the initial amount of drug added was 0.6 % by weight (DTX-A formulation). Non-entrapped DTX was detected in the dispersion media at a concentration of 6 μg/mL. The drug loading and EE of DTX loaded NEs significantly decreased when the initial amount of DTX added was doubled, indicating that the maximum drug loading capacity was reached with the DTX-A formulation. Furthermore, the drug precipitate in the DTX-B formulation was effectively separated from the NE sample, hence further indicating the validity of the dialysis and ultracentrifugation methods for the measurements of drug loading and EE.

As discussed previously, the conjugation of fatty acid to DTX results in a significant improvement in miscibility between the drug and Labrafac™ as well as an increase in drug loading and drug EE in the NE formulations, in comparison to unmodified DTX. According to the results obtained from the ultracentrifugation method, with an additional drug loading of 0.6 % by weight, the percentage of DTX conjugates entrapped in the NE formulations were 92 % (1L-DTX-A formulation) and 97 % (2L-DTX-A formulation). As the initial drug added increased to 5.7 % by weight in the 1L-DTX-B and 2L-DTX-B formulations, approximately 89 % to 91 % of DTX conjugates were entrapped in the NE. The concentration of non-entrapped 2L-DTX and 1L-DTX in the dispersion media was in the range of 1.0 μg/mL to 1.6 μg/mL. Furthermore, small amounts of non-entrapped DTX conjugates (0.06 % to 0.6 % w/w of the added DTX conjugates) were hydrolyzed and converted to DTX in the dispersion media as detected by HPLC analysis. Analysis of the entrapped NE formulations of 1L-DTX and 2L-DTX revealed no DTX. The final concentrations of 1L-DTX and 2L-DTX were 89 mg and
91 mg of DTX equivalent per mL of Labrafac™ in the 1L-DTX-B and 2L-DTX-B formulations, respectively.

Many studies have reported that the drug loading capacity and EE of formulations are related to the solubility and hydrophobicity of the drug. In comparison to DTX, the actual drug loading of the DTX conjugates in the 1L-DTX-B and 2L-DTX-B formulations (Table 3.4) is approximately 10-fold higher in terms of DTX equivalent. This enhancement in the drug loading of the DTX conjugates can be attributed to the increase in the lipophilicity and the miscibility between the DTX conjugates and Labrafac™, in comparison to DTX. The loading of the DTX conjugates in the PEG-based NE formulations is also approximately five times higher than the loading of a PTX-oleate derivative in a cholesterol microemulsion. In the current research, the concentration of drug solubilized was 4.5 mol% (DTX equivalent) relative to the total moles of Labrafac and Solutol HS15, while in the previous study a loading of 0.9 mol% (PTX equivalent relative to total moles of lipid and surfactant) was obtained for PTX-oleate in the cholesterol microemulsion. Furthermore, the loading level of the DTX conjugates in the NE formulations were comparable to the levels achieved for PTX in micelle formulations (i.e. 1% to 4% by weight).

3.4.5. *In vitro* cytotoxicity and hydrolysis of DTX conjugates

In order to maximize drug efficiency, the chemical modification of a taxane to improve solubility should not lead to a pronounced decrease in the activity of the drug at the tumor site. However, many studies have reported that modification at C-2’, C-7 and/or C-10 of taxanes does indeed modify drug activity. In this study, the
cytotoxicity, evaluated by measurement of the 50% inhibitory concentration (IC$_{50}$), of unmodified DTX and DTX conjugates was determined in two cancer cell lines (Table 3.2).

The IC$_{50}$ values for DTX in SKOV3 (1.8 nM) and H460 (1.5 nM) cell lines agreed with previous reports.$^{228,230}$ Unfortunately, the cytotoxic effects of 1L-DTX were much less than those of the parent drug (IC$_{50}$ = 82 or 56 nM; Table 3.2) and the 2L- and 3L-DTX conjugates did not maintain any pharmaceutically relevant cytotoxicity (IC$_{50}$ > 20 μM; Table 3.2). The superior reactivity of the C-2’ group during acyl-chain conjugation to DTX suggests that the ester group at the C-2’ may be more rapidly hydrolyzed than other conjugation points under biological conditions.$^{228,241,242,246}$

Following the 72 hour incubation period in RPMI media, a total of 7 % ± 2 % of the 1L-DTX was hydrolyzed to produce DTX (1 % of DTX) and other taxane derivatives. There was no evidence of hydrolysis of the 2L$_2$;7-DTX and 3L-DTX conjugates.

The proportion of DTX released by hydrolysis of 1L-DTX within 72 hours (1 %) is not large enough to completely account for the activity that 1L-DTX exerted on SKOV3 and H460 cell lines. It remains possible that 1L-DTX conjugate molecules retain some activity that other hydrolysis products were also cytotoxic, or that hydrolysis is increased in the presence of the cell lines. Nevertheless, these studies clearly show that the cytotoxicity of 1L-DTX is significantly impaired in the conjugated form and that this conjugate is slowly hydrolyzed to release the parent drug under biologically relevant conditions.

Similarly, a significant loss in cytotoxicity against the MCF-7 human breast cancer cell line was reported for PTX prodrugs di-substituted at C-2’ and C-7 with acyl
chains, of varying lengths (C6:0 – C16:0) in comparison to PTX (IC\textsubscript{50} < 1nM), due to lack of hydrolysis of the acyl chains\textsuperscript{242}. In contrast, mono-substituted bromoacyl-PTX conjugates (IC\textsubscript{50} values ranged from 3 to 70 nM) were found to retain significantly more of their activity due to ease of hydrolysis of the bromoacyl chains\textsuperscript{242}. In vitro, PTX prodrugs modified with shorter bromoacyl chains (i.e. C6:0 – C12:0) were found to be more active than PTX conjugates with longer bromoacyl chains (i.e. C14:0 - C16:0). In contrast, in vivo it was the formulation including 2'-(2-bromo)-hexadecanoyl (C16:0, IC\textsubscript{50} = 70 nM) that was most efficacious, in comparison to liposome formulations incorporating the PTX conjugates with shorter chain bromoacyl moieties\textsuperscript{242}. The authors put forth that the superior efficacy of the formulation incorporating the longer chain bromoacyl PTX conjugate suggests that “slow but sustained delivery” of PTX to tumor cells is advantageous in vivo\textsuperscript{242}. In light of this information, the 1L-DTX prodrug in combination with the non-toxic NE formulation, that was shown to circulate for prolonged periods in vivo and accumulate preferentially at tumor sites, may be a promising combination\textsuperscript{106, 109}

3.5. Conclusion

This study demonstrates that conjugation of DTX to a moiety that is chemically similar to that of the solubilizing media of the formulation is a promising strategy for enhancing the drug loading and encapsulation efficiency in a NE formulation. However, these studies also highlight the need to consider the impact of chemical modification of a drug on biological activity. Theoretical calculations predicted that the DTX-lauroyl conjugates would have increased solubility in Labrafac\textsuperscript{TM} with the tri-substitute being
favored over the di- and mono- substitutes. Indeed, experimental evaluation revealed that synthesis of the mono, di and tri-substituted conjugates resulted in marked increases in the solubility and loading of the drug (in terms of DTX equivalents) in the NE formulation. However, a balance must be achieved between optimizing solubility and maintaining activity. In this regard, the mono-substituted conjugate (1L-DTX) is identified as the DTX prodrug with the requisite properties for further consideration.

3.6. Acknowledgements

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding this research. JCL acknowledges the Steacie Fellowship from the NSERC and support from the Canada research chair program. L. Huynh thanks Chris Neale, for useful discussions regarding the *in vitro* cell culture studies, and Gattefossé Canada, Inc. for supplying Labrafac™. Technical assistance from Angel Fu and Laurence Luk is also acknowledged.
Chapter 4: Systematic Design of Unimolecular Star Copolymer Micelles using Molecular Dynamics Simulations

The work described in this chapter has been published in the following reference:


Abstract: Star copolymers (SCPs) have recently attracted considerable attention due to their unique applicability in a wide range of biomedical fields. With the intention of rationally designing a stable unimolecular SCP, atomistic molecular dynamics simulations of thirteen SCPs are conducted. The SCPs each have six identical arms of methoxypoly(ethylene glycol)-b-polycaprolactone (MePEG\textsubscript{x}-b-PCL\textsubscript{y}) and systematically vary in terms of total molecular weight and ratio of hydrophobic to hydrophilic block length. For all hydrated SCPs, the simulations predict a densely packed hydrophobic PCL core that excludes water and is phase separated from a highly mobile hydrophilic PEG corona. The radii of the hydrophobic PCL core and the PEG blocks are independent of each other and can be predicted over a broad molecular weight range. A linear relationship between the hydration and the molecular weight of the PEG blocks is observed with the average number of water molecules bound per PEG repeat unit within the range of that determined experimentally. As well, a quantitative relationship relates the water accessible surface area of the hydrophobic PCL core to the molecular weights of PCL and PEG moieties. We postulate that the propensity for aggregation of SCPs into multimolecular micelles is correlated with the partial hydration of the hydrophobic core of unimers. Our results suggest that SCPs with a hydrophobic PCL core \( \leq 2 \text{ kDa per arm} \) are fully protected from water when the hydrophilic PEG blocks approach 14.6 kDa per arm. We therefore predict that SCPs of this composition yield unimolecular micelles that are thermodynamically stable at low concentrations.
4.1. Introduction

Linear, amphiphilic diblock and triblock copolymers have emerged as the materials of choice for use in a wide range of biomedical applications, including fabrication or coating of biomedical devices, drug delivery, and tissue engineering.\textsuperscript{126-129} The use of block copolymers in these technological platforms brings unparalleled diversity since they can be synthesized such that they self-organize or self-assemble, under specific conditions, to form superstructures with dimensions on the order of the nano- or micrometer.\textsuperscript{247} Self-organization of these materials into ordered structures is reliant on the presence of specific intra- or intermolecular interactions. Understanding the relationship between the composition of these materials and the self-organized superstructures they form is necessary for their rational design and use in particular applications.

Recent advances in synthetic procedures have afforded controlled preparation of block copolymers having complex architectures, such as dendrimer-like, graft-block, or star-block copolymers (SCPs).\textsuperscript{138, 139, 248} In aqueous media amphiphilic SCPs, with central hydrophobic blocks surrounded by terminal hydrophilic blocks, can be used for the solubilization or delivery of hydrophobic solutes.\textsuperscript{249, 250} The hydrophobic core-forming blocks serve as cargo space for the lipophilic solutes while the hydrophilic blocks form a shell that protects the core from the aqueous environment. SCPs can form unimolecular or multimolecular micelles depending on the copolymer composition and architecture.\textsuperscript{137, 249, 251} Of particular interest, SCPs that form unimolecular micelles are not faced with issues relating to thermodynamic instability and may be prepared to be smaller in size with a more narrow size distribution than most multimolecular systems.\textsuperscript{135, 136} To date,
numerous SCP systems have been put forth in an attempt to design unimolecular micelles. These SCPs include star amphiphilic block copolymers based on poly(ethylene glycol)-b-polycaprolactone (PEG-b-PCL) and PEG-b-poly(L-lactide).\textsuperscript{249, 251} In only a few cases, however, have the solution properties of these systems been studied thoroughly.\textsuperscript{137, 249, 251} As such, there remains a limited understanding of the relationship between the composition and architecture of SCPs (such as the number of arms, the nature and length of hydrophobic/hydrophilic blocks and the total molecular weight), their self-organizing behavior, and the properties of the unimolecular or multimolecular micelles that they form. As well, few studies have examined the structure, the hydration state of the inner hydrophobic core and outer hydrophilic corona-forming blocks, and the composition of the core-corona interface in SCP systems. These parameters influence important properties of the micelles, such as thermodynamic and kinetic stability, degradation profile, and solute loading capacity.

Computational methods have recently been used to study the behaviour of SCPs in unimolecular and multimolecular states.\textsuperscript{50, 61, 140, 252} However, the solution behaviors of these copolymers and their intermolecular interactions have not previously been explored in atomistic detail. This is likely due to the fact that significant computational resources are required for these large systems. The aim of our current study is to gain fundamental insight into the properties of SCPs in aqueous solution and to rationally design a stable unimolecular star copolymer by conducting all-atom molecular dynamics (MD) simulation studies of these systems in explicit water. The atomistic resolution of the simulations enables explicit treatment of the physical basis of molecular self-aggregation, including the interactions that underlie the hydrophobic effect. As a first step towards the
rational/structure-based design of unimolecular micelles, in the present study the solution properties of differently composed SCPs as unimers are evaluated by MD simulation. Atomistic simulations of thirteen SCPs in water were carried out for a total simulation time of 200 ns for each system. The present study yields insight into the equilibrium conformations of the SCPs as well as detailed information on the hydrophobic PCL and hydrophilic PEG blocks, and their interactions with water, during self-organization. In light of the potential benefits of monodisperse unimolecular micelles, our study is focused largely on predicting the parameters that are likely to influence the equilibrium governing copolymer aggregation and micelle disassembly, indirectly probing the mechanism of aggregation. Dynamic and static light scattering revealed that six-armed \([\text{PCL}_{18}-b-\text{PEG}_{113}]_6\) SCPs aggregate, forming multimolecular micelles in water (F. Li and C. Allen, unpublished results). The molecular basis of this unimeric instability is, however, difficult to assess experimentally due to the low concentrations required to obtain the unimeric state. In contrast, molecular simulations permit the study of unimeric states in isolation. Here, we take advantage of this capability to examine the relationship between SCP composition and solvation, and to quantify the exposed hydrophobic surface area of the core. PEG coronas that completely protect the hydrophobic core from solvent during unimeric simulation are likely to displace the equilibrium governing aggregation toward the unimeric state.

4.2. Methodology

4.2.1. Simulated systems
The series of SCPs under investigation have six identical arms radiating from a small central core. The arms are composed of methoxyPEG (MePEG) and PCL (i.e. $[\text{MePEG}_x-b-\text{PCL}_y]^6$, Table 4.1). The thirteen systems simulated contain a single hydrated $[\text{MePEG}_x-b-\text{PCL}_y]^6$ SCP and vary systematically in terms of the degree of polymerization of the PEG, $N_{\text{PEG}}$, and PCL, $N_{\text{PCL}}$, in each arm (i.e. number of repeat units, x and y, respectively) as well as in terms of total MW of the SCP and $N_{\text{PCL}}:N_{\text{PEG}}$ ratio. The composition and size of each system used in the MD simulations is outlined in Table 4.1.
Table 4.1: Composition of the [MePEG$_x$-$b$-PCL$_y$]$_6$ star copolymer systems used in the molecular dynamics simulations.

<table>
<thead>
<tr>
<th>x-y</th>
<th>x:y ratio</th>
<th>Volume $^a$ (nm$^3$)</th>
<th>number of water molecules</th>
<th>MW$_{PEG}$ per arm</th>
<th>MW$_{PCL}$ per arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-1</td>
<td>6.0</td>
<td>164</td>
<td>5194</td>
<td>295</td>
<td>114</td>
</tr>
<tr>
<td>12-2</td>
<td>6.0</td>
<td>209</td>
<td>6566</td>
<td>560</td>
<td>228</td>
</tr>
<tr>
<td>19-2</td>
<td>9.5</td>
<td>349</td>
<td>11053</td>
<td>868</td>
<td>228</td>
</tr>
<tr>
<td>19-3</td>
<td>6.3</td>
<td>333</td>
<td>10482</td>
<td>868</td>
<td>342</td>
</tr>
<tr>
<td>27-2</td>
<td>13.5</td>
<td>504</td>
<td>16021</td>
<td>1221</td>
<td>228</td>
</tr>
<tr>
<td>27-3</td>
<td>9.0</td>
<td>526</td>
<td>16634</td>
<td>1221</td>
<td>342</td>
</tr>
<tr>
<td>27-4</td>
<td>6.8</td>
<td>476</td>
<td>15023</td>
<td>1221</td>
<td>456</td>
</tr>
<tr>
<td>38-3</td>
<td>12.7</td>
<td>512</td>
<td>17412</td>
<td>1705</td>
<td>342</td>
</tr>
<tr>
<td>38-4</td>
<td>9.5</td>
<td>504</td>
<td>15778</td>
<td>1705</td>
<td>456</td>
</tr>
<tr>
<td>38-6</td>
<td>6.3</td>
<td>493</td>
<td>15335</td>
<td>1705</td>
<td>684</td>
</tr>
<tr>
<td>38-9</td>
<td>4.2</td>
<td>557</td>
<td>17365</td>
<td>1705</td>
<td>1026</td>
</tr>
<tr>
<td>113-18</td>
<td>6.3</td>
<td>3389</td>
<td>108338</td>
<td>5010</td>
<td>2052</td>
</tr>
<tr>
<td>118-6</td>
<td>19.7</td>
<td>2797</td>
<td>89429</td>
<td>5230</td>
<td>684</td>
</tr>
</tbody>
</table>

$x$ and $y$ are the number of repeat units in the PEG and PCL blocks, respectively, of each arm in the [MePEG$_x$-$b$-PCL$_y$]$_6$ star copolymers. ($^a$) volume of the simulation box.
4.2.2. Star block copolymer parameters

The parameters for Lennard-Jones interactions and Ryckaert-Bellemans dihedrals applied to the [MePEG<sub>x</sub>-<i>b</i>-PCL)<sub>y</sub>]<sub>6</sub> SCRs are taken from the optimized potentials for liquid simulations (OPLS) parameter set<sup>253</sup>, <sup>254</sup> and from Charifson <i>et al.</i> for PCL ester bond angle.<sup>255</sup> The parameters for the CH<sub>2</sub>OCOOCH<sub>2</sub> fragment that connects PCL to PEG are adapted from those of dimethyl carbonate<sup>256</sup> as outlined in Table 4.2. A complete list of SCP charge assignments is included in Table 4.3. The simple point charge (SPC) water model is used as explicit solvent.<sup>257</sup> Previously, studies have shown that OPLS-AA and other force fields in conjunction with different water models, including SPC and TIP4P, yield free energies of solvation with similar accuracy.<sup>258</sup>, <sup>259</sup>
Table 4.2: Parameters for the CH$_2$OCOOCH$_2$ fragment.

![Chemical structure of CH$_2$OCOOCH$_2$ fragment]

<table>
<thead>
<tr>
<th>Atom types</th>
<th>mass (g·mol$^{-1}$)</th>
<th>charge (e)</th>
<th>$\sigma$ (nm)</th>
<th>$\varepsilon$ (kJ·mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_D$</td>
<td>15.99940</td>
<td>-0.505</td>
<td>0.296000</td>
<td>0.878640</td>
</tr>
<tr>
<td>O$_B$</td>
<td>15.99940</td>
<td>-0.460</td>
<td>0.300000</td>
<td>0.711280</td>
</tr>
<tr>
<td>C$_C$</td>
<td>12.01100</td>
<td>0.799</td>
<td>0.375000</td>
<td>0.439320</td>
</tr>
<tr>
<td>C$_A$</td>
<td>12.01100</td>
<td>0.253</td>
<td>0.350000</td>
<td>0.276144</td>
</tr>
<tr>
<td>H$_A$</td>
<td>1.00800</td>
<td>0.003</td>
<td>0.242000</td>
<td>0.062760</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond types</th>
<th>Bond length (nm)</th>
<th>Bond stretching force constant (kJ·mol$^{-1}$·nm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_D$ C$_C$</td>
<td>0.12169</td>
<td>381999.0</td>
</tr>
<tr>
<td>C$_C$ O$_B$</td>
<td>0.13279</td>
<td>220497.0</td>
</tr>
<tr>
<td>O$_B$ C$_A$</td>
<td>0.14487</td>
<td>141001.0</td>
</tr>
<tr>
<td>H$_A$ C$_A$</td>
<td>0.10900</td>
<td>284512.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angle types</th>
<th>Angle (rad)</th>
<th>Angle bending force constant (kJ·mol$^{-1}$·rad$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_B$ C$_C$ O$_B$</td>
<td>110.4</td>
<td>429.2784</td>
</tr>
<tr>
<td>O$_D$ C$_C$ O$_B$</td>
<td>124.8</td>
<td>430.5336</td>
</tr>
<tr>
<td>C$_C$ O$_B$ C$_A$</td>
<td>119.2</td>
<td>348.1088</td>
</tr>
<tr>
<td>O$_B$ C$_A$ H$_A$</td>
<td>109.5</td>
<td>292.8800</td>
</tr>
<tr>
<td>H$_A$ C$_A$ H$_A$</td>
<td>107.8</td>
<td>276.1440</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dihedral Angle</th>
<th>C$_1$ (kJ·mol$^{-1}$)</th>
<th>C$_2$ (kJ·mol$^{-1}$)</th>
<th>C$_3$ (kJ·mol$^{-1}$)</th>
<th>C$_4$ (kJ·mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_A$ O$_B$ C$_C$ O$_D$</td>
<td>21.43881</td>
<td>0</td>
<td>21.43881</td>
<td>0</td>
</tr>
<tr>
<td>C$_A$ O$_B$ C$_C$ O$_B$</td>
<td>16.15810</td>
<td>1.683820</td>
<td>-14.7337</td>
<td>-3.10824</td>
</tr>
<tr>
<td>H$_A$ C$_A$ O$_B$ C$_C$</td>
<td>0.41421</td>
<td>1.242650</td>
<td>0</td>
<td>-1.65686</td>
</tr>
</tbody>
</table>

(a) The H$_A$C$_A$H$_A$ angle type was obtained from the standard OPLS-AA of alkanes.$^{19,20}$ (b) Dihedral angle constants C$_5$ and C$_6$ = 0. All other parameters were adapted from Okada et al.$^{22}$
Table 4.3: Charge assignments for the residues of [MePEG\textsubscript{x}-b-PCL\textsubscript{y}]\textsubscript{6} star copolymers.

<table>
<thead>
<tr>
<th>residues</th>
<th>atoms</th>
<th>charge (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(ethylene glycol) (PEG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{A}</td>
<td>-0.400</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{B}</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{B}</td>
<td>0.030</td>
</tr>
<tr>
<td>polycaprolactone (PCL)</td>
<td>C\textsubscript{A}</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{A}</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{B}</td>
<td>-0.330</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{C}</td>
<td>0.510</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{D}</td>
<td>-0.120</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{E}</td>
<td>-0.430</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{D}</td>
<td>0.060</td>
</tr>
<tr>
<td>terminal group: CH\textsubscript{2}OCH\textsubscript{3}</td>
<td>C\textsubscript{A}</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{A}</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{B}</td>
<td>-0.400</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{C}</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{C}</td>
<td>0.030</td>
</tr>
<tr>
<td>central core: (CCH\textsubscript{2})O</td>
<td>C\textsubscript{A}</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{B}</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{C}</td>
<td>-0.400</td>
</tr>
<tr>
<td></td>
<td>H\textsubscript{B}</td>
<td>0.030</td>
</tr>
</tbody>
</table>

The dash bond is the proximal and/or distal end that connects to other residue(s). Charge assignments for the CH\textsubscript{2}OCOOCH\textsubscript{2} residue (connection fragment between the PEG and PCL) are shown in Table 4.2.
4.2.3. Molecular dynamics simulation protocol

The extended initial coordinates for the [MePEG_x-b-PCL_y]_6 SCPs are generated using Cerius² 4.6 software (Accelrys Inc., San Diego, CA.). MD simulations are performed using the leap-frog algorithm for integrating Newton’s equations of motion with the GROMACS 3.3.1 simulation software.²⁶⁰,²⁶¹ A cubic box containing water and one solute molecule is constructed with an initial minimum distance of 10 Å between the solute and the boundary. The particle-mesh Ewald (PME) method is used to calculate the electrostatic interactions every time step with a real-space cutoff of 9 Å. Lennard-Jones interactions are computed using the group-based twin-range cutoff method, calculating interactions every step for separation distances less than 9 Å and every ten steps for separation distances less than 14 Å, when the nonbonded list is updated. The LINCS algorithm is applied to constrain the bond lengths of the solute and the SETTLE algorithm is applied to water molecules to constrain their internal geometry. Following 5000 steps of steepest-descent energy minimization, a short MD simulation is performed in the NPT ensemble at a constant pressure of 1 bar and a temperature of 27ºC, while applying position restraints to the solute. The pressure is controlled isotropically by a Berendsen barostat with a coupling time constant of 4.0 ps⁻¹. To control the temperature, the solute and the solvent are separately coupled to Berendsen thermostats with coupling constants of 0.1 ps⁻¹. Production dynamics are conducted for 200 ns with an integration time step of 2 fs. The coordinates are stored every 10 ps.
4.2.4. Analysis

**Structure:** Structural analyses are performed by utilizing analysis tools from GROMACS 4.0.4. The average radius of gyration (Rg) of the hydrophobic PCL core, Rgcore, is determined based on all PCL blocks and the dipentaerythriol initiator, whereas the average Rg of the hydrophilic PEG, RgPEGarm, is calculated for each PEG arm of the SCP and then averaged. The asphericity index of the hydrophobic PCL core is determined according to the method described by Bruns and Carl (Appendix B) where the asphericity index is zero for a perfect sphere and approaches one as the degree of asphericity increases.

**Hydration:** The spatial range of first hydration shell of PEG is determined based on the first minimum of the radial distribution function (RDF) of all water atoms around the terminal carbon atom of each PEG arm (3.5 Å). The total number of water molecules within the first hydration shell of the six PEG arms excluding the six OCH₃ terminal groups, WPEG, is calculated and then used to determine the average number of water molecules bound per PEG repeat unit (WperPEG). The solvent accessible surface area of the PEG blocks excluding the six OCH₃ terminal groups (APEG) for the SCPs in aqueous solution is evaluated using the algorithm of Connolly. The average surface area of a water molecule in contact with the polymer, A₃H₂O (10.2 Å²), is obtained from the APEG:WPEG ratio for the smallest simulation system. The number of water molecules within 3.5 Å of the PCL core, WPCL, is multiplied by A₃H₂O in order to obtain the solvent accessed surface area of the PCL core, SASPCL. Similarly, the solvent accessed surface area of the PEG blocks, SASPEG, is determined based on WPEG and A₃H₂O. In cases where a water molecule interacts simultaneously with PEG and PCL polymers, one-half of the
interaction is assigned to each polymer. Furthermore, the total solvent accessible surface area of the hydrophobic core ($A_{\text{totPCL}}$) is calculated after removing the PEG blocks and water from the trajectory and then applying the algorithm of Connolly.\textsuperscript{171} Therefore, the difference between $A_{\text{totPCL}}$ and $\text{SAS}_{\text{PCL}}$ is the surface area of PCL that is protected from water by the PEG blocks ($A_{\text{protectedPCL}}$). It is worth noting that the solvent accessed surface area is the surface area that is actually in contact with water molecules during the simulation, whereas the solvent accessible surface is the area that is available for potential contact by water molecules during simulation and is calculated based on the model developed by Connolly.\textsuperscript{171} The various properties calculated for the [MePEG\textsubscript{x-b-PCL\textsubscript{y}}]\textsubscript{6} SCPs are outlined in Figure 4.1.
Figure 4.1: Summary of properties calculated for [MePEG₅₋b-PCL₃]₆ star copolymers. (a) PEG (blue) is shown partially protecting PCL (yellow). $R_g^{\text{PEG arm}}$ and $EED_{\text{PEG arm}}$ are calculated for each PEG arm and then averaged. $SAS_{\text{PEG}}$, $W_{\text{PEG}}$ and $R_g^{\text{star}}$ are calculated using the entire SCP. $W_{\text{perPEG}}$ is calculated for each PEG repeat unit and then averaged. (b) The PCL core of the SCP is shown without its associated PEG blocks. Water molecules within 3.5 Å of PCL are shown explicitly and counted to yield $W_{\text{PCL}}$. The area of PCL in contact with water (red) is calculated as $SAS_{\text{PCL}} = W_{\text{PCL}} \times A_{\text{H}_2\text{O}}$, while the PCL that is successfully protected from water by PEG (yellow) is calculated as $A_{\text{protectedPCL}} = A_{\text{totPCL}} - SAS_{\text{PCL}}$. The total PCL surface area (yellow + red) is represented by $A_{\text{totPCL}}$. The fraction of the PCL surface area that is protected from water by the PEG blocks, $f_{\text{protectedPCL}}$, is calculated as $A_{\text{protectedPCL}}$ divided by $A_{\text{totPCL}}$. (c) Two PCL cores with different asphericity values are shown to assist the reader interpret this metric.
4.3. Results

4.3.1. Structural properties

**Equilibration and convergence:** The SCPs investigated in this study are composed of six MePEG<sub>x</sub>-<i>b</i>-PCL<sub>y</sub> arms covalently attached through a small central core, as shown in Table 4.1. Snapshots of the conformation of [MePEG<sub>38</sub>-<i>b</i>-PCL<sub>9</sub>]<sub>6</sub> following 1 ns and 200 ns of simulation are shown in Figure 4.2a. The hydration of [MePEG<sub>38</sub>-<i>b</i>-PCL<sub>9</sub>]<sub>6</sub> as a function of simulation time is shown in Figure 4.2b. According to W<sub>PEG</sub> and W<sub>PCL</sub>, the solvent interaction of the simulated systems is converged after 40 ns (Appendix B). Also, 40 ns of simulation is sufficient to converge R<sub>gcore</sub>, R<sub>garm</sub> (Appendix B). Generally, during the MD simulations, the PCL blocks quickly collapse and form a compact core within 15 ns. The hydrophilic PEG blocks remain highly hydrated and assume disordered conformations with a high degree of structural heterogeneity.
**Figure 4.2:** (a) Snapshot of the [MePEG\textsubscript{38}-b-PCL\textsubscript{9}]\textsubscript{6} star copolymer at 1 ns and 200 ns highlighting the conformations of PCL (yellow) and PEG (blue) blocks, and water molecules within 3.5 Å of PCL. Bulk water and hydrogen atoms are omitted for clarity. (b) Hydration of PCL core (♦) and PEG blocks (◊) and Rg of PCL core (▲) and PEG blocks (△) of the [MePEG\textsubscript{38}-b-PCL\textsubscript{9}]\textsubscript{6} star copolymer as a function of simulation time.
Self-aggregated structure, size, and shape of PCL: During MD simulations, the PCL blocks form a compact hydrophobic core that preferentially excludes water (data not shown). Concurrently, strong segregation occurs between the hydrophobic PCL core and the hydrophilic PEG corona, as exemplified by the snapshot of [MePEG$_{113}$-b-PCL$_{18}$]$_6$ in Figure 4.3a.

**Figure 4.3:** Spatial distribution of the PCL core and PEG corona in [MePEG$_{113}$-b-PCL$_{18}$]$_6$ (a) Cross section through a randomly selected but representative snapshot showing the PEG corona (blue) and the PCL core (yellow) (b) Density profile of PCL core (yellow), PEG blocks (blue) and water (black) as a function of distance from the center of mass of the PCL core averaged over the last 160 ns of molecular dynamics simulation.
Importantly, self-organization of the hydrophobic PCL core is observed. The hydrophobic cores of SCPs with $N_{PCL} \geq 9$ (i.e. with $y = 9$ or 18) are relatively spherical, having an asphericity index of $\leq 0.009 \pm 0.001$ (Table 4.4). As $N_{PCL}$ decreases, the shape of the hydrophobic core shifts towards an ellipsoid where the asphericity index of the core ranges from 0.017 to 0.049 as shown in Table 4.4. Amongst the different systems, the interior hydrophobic PCL core becomes more densely packed with increasing total $MW_{PCL}$ and reaches a density of 1.1 to 1.2 $g/cm^3$ at a distance of 4 Å from the center of mass (COM) of the hydrophobic core (Appendix B).

As shown in Figure 4.4a, the average $R_{gcore}$ can be described as a function of $N_{PCL}$ according to the following equation:

$$R_{gcore} = 6.05 \ N_{PCL}^{0.273} \ (Å). \ \ \ \ \ (R^2 = 0.986) \ \ \ \ \ (4.1)$$
Table 4.4: Asphericity and average radius of gyration of [MePEG\textsubscript{x-b-PCL\textsubscript{y}}\textsubscript{6} star copolymers computed from the last 160 ns of the molecular dynamics simulation.

<table>
<thead>
<tr>
<th>x-y</th>
<th>R\textsubscript{g}\textsubscript{star} (Å)</th>
<th>R\textsubscript{g}\textsubscript{PEGarm} (Å)</th>
<th>R\textsubscript{g}\textsubscript{core} (Å)</th>
<th>Asphericity x10\textsuperscript{-3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-1</td>
<td>9.4 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>9.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-2</td>
<td>11.8 ± 0.5</td>
<td>6.3 ± 0.3</td>
<td>7.3 ± 0.4</td>
<td>9.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td>19-2</td>
<td>13.5 ± 0.6</td>
<td>8.0 ± 0.4</td>
<td>7.4 ± 0.5</td>
<td>9.3 ± 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2 ± 2.4</td>
</tr>
<tr>
<td>19-3</td>
<td>13.2 ± 0.6</td>
<td>8.0 ± 0.5</td>
<td>8.2 ± 0.4</td>
<td>9.4 ± 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>27-2</td>
<td>15.0 ± 0.9</td>
<td>9.7 ± 0.5</td>
<td>7.7 ± 0.3</td>
<td>9.3 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>4.9 ± 2.1</td>
</tr>
<tr>
<td>27-3</td>
<td>14.9 ± 0.7</td>
<td>9.3 ± 0.6</td>
<td>8.4 ± 0.3</td>
<td>8.2 ± 3.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>27-4</td>
<td>14.9 ± 0.7</td>
<td>9.6 ± 0.6</td>
<td>8.7 ± 0.3</td>
<td>8.7 ± 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>38-3</td>
<td>17.0 ± 0.9</td>
<td>10.8 ± 0.7</td>
<td>8.1 ± 0.4</td>
<td>8.4 ± 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>38-4</td>
<td>16.6 ± 1.0</td>
<td>11.0 ± 0.5</td>
<td>8.7 ± 0.2</td>
<td>7.8 ± 2.4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>38-6</td>
<td>16.0 ± 0.6</td>
<td>11.0 ± 0.6</td>
<td>9.9 ± 0.3</td>
<td>8.2 ± 2.6</td>
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<tr>
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<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>38-9</td>
<td>16.1 ± 0.9</td>
<td>11.5 ± 0.7</td>
<td>10.9 ± 0.5</td>
<td>8.7 ± 2.8</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>113-18</td>
<td>24.1 ± 1.6</td>
<td>16.8 ± 0.7</td>
<td>13.3 ± 0.2</td>
<td>9.3 ± 3.0</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>118-6</td>
<td>26.4 ± 1.6</td>
<td>19.8 ± 0.3</td>
<td>9.7 ± 0.2</td>
<td>6.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.7 ± 0.6</td>
</tr>
</tbody>
</table>

x and y are the number of repeat units in the PEG and PCL blocks, respectively, of each arm in the [MePEG\textsubscript{x-b-PCL\textsubscript{y}}\textsubscript{6} star copolymers. R\textsubscript{g}\textsubscript{core} and R\textsubscript{g}\textsubscript{star} are the radius of gyration of the PCL core (PCL and dipentaerythriol initiator) and entire star copolymer, respectively. R\textsubscript{g}\textsubscript{PEGarm} is the average radius of gyration of each PEG arm. Values in parentheses are the radii of gyration for the PEG blocks calculated based on Flory’s model \textsuperscript{55,56}, R\textsubscript{gFloryPEG} = (a/\sqrt{6})N\textsubscript{PEG}\textsuperscript{α}, where a = 2.8 Å for PEG \textsuperscript{57} and α = 0.588 for polymer in good solvent.
Figure 4.4: Double-logarithmic scales of the mean radius of gyration of (a) the PCL core (R_{g,core}) versus N_{PCL} (●), the PEG block (R_{g,PEGarm}) versus N_{PEG} (○) and (b) the entire star copolymer (R_{g,star}) versus sum of N_{PCL}^{0.273} and N_{PEG}^{0.479} for [MePEG_y-b-PCL_x]_6 star copolymers. Data are computed from the last 160 ns of the molecular dynamics simulations. The dashed lines are the linear fit functions. The radius of gyration is expressed in Å.
Transition phase between PCL and PEG: There exists a distance from the COM of the core at which the density of PCL equals that of PEG, generally in the presence of a small but reproducible amount of water (Figure 4.3b). This represents the most likely distance from the COM of the core at which an amphiphilic transition phase exists between the dominantly hydrophobic and hydrophilic regions of the aggregated copolymer. The apparent thickness of this transition (Figure 4.3b) is an artifact that results from radially averaging an imperfect spherical object. The environmental transition is nearly discrete when instantaneous snapshots are assessed (Figure 4.3a).

Conformation, size, and extension of PEG: In contrast to PCL, the PEG blocks are highly hydrated during the MD simulation, and thus the PEG corona is significantly less densely packed than the hydrophobic PCL core (Figure 4.3b). As such, the PEG corona occupies a significantly larger volume than the core. For all SCPs investigated in the current study, the MD simulations reveal that the PEG blocks move rapidly and are globally disordered while transiently adopting a local helical structure with 3.5 PEG repeat units per turn (data not shown). For SCPs with \( N_{\text{PEG}} \geq 19 \), the distal ends of the PEG blocks either extend away from the proximal ends, or curl back to form a Gaussian coil providing partial shielding of the hydrophobic core from solvent as shown in the trajectory of \([\text{MePEG}_{113-b-\text{PCL}_{18}}]_6\). For SCPs with shorter PEG chains, the ends of all PEG blocks remain extended, with a median \( \text{EED}_{\text{PEGarm}} \) of 8.0 ± 0.1 Å (\( N_{\text{PEG}} = 6 \)) and 10.5 ± 0.1 Å (\( N_{\text{PEG}} = 12 \)) as shown in Appendix B). The conformation of PEG in the corona is also measured by the Rg of each PEG arm, \( Rg_{\text{PEGarm}} \). A linear relationship is obtained for \( Rg_{\text{PEGarm}} \) as a function of \( N_{\text{PEG}} \) (Figure 4.4a), allowing \( Rg_{\text{PEGarm}} \) to be predicted using the following relationship:
\[
R_{gPEGarm} = 1.93 \, N_{PEG}^{0.479} \, (\text{Å}) \quad \quad (R^2 = 0.989) \quad (4.2)
\]

As shown in Figure 4.4b, the size of the SCP is more strongly related to \( N_{PEG} \) than to \( N_{PCL} \) and can be determined based on the relationship between the \( R_g \) of the entire SCP, \( R_{g\text{star}} \), and the total degree of polymerization of the PEG and PCL blocks as shown in Equation 4.3 (Table 4.4 and Figure 4.4b).

\[
R_{g\text{star}} = 3.59 \, (N_{PEG}^{0.479} + N_{PCL}^{0.273})^{0.780} \, (\text{Å}) \quad (R^2 = 0.975) \quad (4.3)
\]

### 4.3.2. Solvation properties

Hydration and solvent accessible surface area of PCL: MD simulation of SCPs in explicit water allows the calculation of the number of water molecules in the first hydration shell (within 3.5 Å) of the polymers. A snapshot of the \([\text{MePEG}_{38-b-PCL}_{9}]_6\) SCP highlighting the interaction of the hydrophobic PCL core with water at a simulation time of 200 ns is shown in Figure 4.1b. Once the PCL component exceeds a total \( MW_{PCL} \) of 2.05 kDa \((N_{PCL} = 3)\), the interior of the hydrophobic core is completely devoid of water (data not shown). Importantly, the number of water molecules bound to the surface of the hydrophobic PCL core, \( W_{PCL} \), is found to depend on both \( N_{PCL} \) and \( N_{PEG} \). In particular, \( W_{PCL} \) decreases with an increase in \( N_{PEG} \) when \( N_{PCL} \) is held constant, as shown in Table 4.5. The quantification of this relationship, as described below, is one of the major results in this study, and is an essential step towards the rational design of unimolecular SCP micelles.
Table 4.5: Solvation properties for the PEG and PCL blocks of star copolymers computed from the last 160 ns of the molecular dynamics simulation.

<table>
<thead>
<tr>
<th>x-y</th>
<th>$W_{\text{PEG}}$ a</th>
<th>$W_{\text{PCL/PEG}}$</th>
<th>$W_{\text{PCL}}$</th>
<th>$\text{SAS}_{\text{PEG}}$ ($\text{Å}^2$)</th>
<th>$\text{SAS}_{\text{PCL}}$ ($\text{Å}^2$)</th>
<th>$A_{\text{totPCL}}$ ($\text{Å}^2$)</th>
<th>$A_{\text{protectedPCL}}$ ($\text{Å}^2$) b</th>
<th>$f_{\text{protectedPCL}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-1</td>
<td>177 ± 6 (4.9)</td>
<td>32 ± 2</td>
<td>43 ± 2</td>
<td>1888 ± 74</td>
<td>461 ± 23</td>
<td>1129 ± 31</td>
<td>669 ± 38</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>12-2</td>
<td>317 ± 11 (4.4)</td>
<td>45 ± 3</td>
<td>57 ± 4</td>
<td>3366 ± 140</td>
<td>614 ± 44</td>
<td>1689 ± 69</td>
<td>1075 ± 82</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>19-2</td>
<td>467 ± 23 (4.1)</td>
<td>45 ± 4</td>
<td>46 ± 4</td>
<td>4968 ± 268</td>
<td>488 ± 43</td>
<td>1704 ± 63</td>
<td>1216 ± 76</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>19-3</td>
<td>448 ± 23 (3.9)</td>
<td>55 ± 4</td>
<td>62 ± 5</td>
<td>4762 ± 271</td>
<td>657 ± 52</td>
<td>2169 ± 68</td>
<td>1512 ± 101</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>27-2</td>
<td>616 ± 27 (3.8)</td>
<td>49 ± 7</td>
<td>39 ± 5</td>
<td>6550 ± 310</td>
<td>418 ± 57</td>
<td>1871 ± 67</td>
<td>1453 ± 88</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>27-3</td>
<td>611 ± 27 (3.8)</td>
<td>57 ± 6</td>
<td>53 ± 5</td>
<td>6498 ± 313</td>
<td>559 ± 55</td>
<td>2444 ± 77</td>
<td>1885 ± 95</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>27-4</td>
<td>617 ± 37 (3.8)</td>
<td>62 ± 5</td>
<td>60 ± 5</td>
<td>6559 ± 431</td>
<td>640 ± 48</td>
<td>2564 ± 76</td>
<td>1924 ± 90</td>
<td>0.75 ± 0.04</td>
</tr>
<tr>
<td>38-3</td>
<td>860 ± 40 (3.8)</td>
<td>53 ± 6</td>
<td>38 ± 4</td>
<td>9146 ± 449</td>
<td>405 ± 45</td>
<td>2143 ± 82</td>
<td>1738 ± 93</td>
<td>0.81 ± 0.05</td>
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<tr>
<td>38-4</td>
<td>816 ± 45 (3.6)</td>
<td>62 ± 8</td>
<td>47 ± 6</td>
<td>8679 ± 501</td>
<td>500 ± 67</td>
<td>2606 ± 75</td>
<td>2106 ± 101</td>
<td>0.81 ± 0.05</td>
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<td>38-6</td>
<td>797 ± 40 (3.5)</td>
<td>82 ± 8</td>
<td>64 ± 6</td>
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<td>682 ± 64</td>
<td>3421 ± 112</td>
<td>2740 ± 129</td>
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<td>38-9</td>
<td>794 ± 29 (3.5)</td>
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<td>93 ± 4</td>
<td>8445 ± 322</td>
<td>988 ± 79</td>
<td>4359 ± 112</td>
<td>3371 ± 165</td>
<td>0.77 ± 0.04</td>
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<tr>
<td>113-18</td>
<td>2206 ± 124 (3.3)</td>
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<td>88 ± 6</td>
<td>23468 ± 1329</td>
<td>932 ± 63</td>
<td>7239 ± 168</td>
<td>6307 ± 179</td>
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</tr>
<tr>
<td>118-6</td>
<td>2495 ± 107 (3.5)</td>
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<td>40 ± 4</td>
<td>26533 ± 1166</td>
<td>442 ± 47</td>
<td>3292 ± 109</td>
<td>2868 ± 119</td>
<td>0.87 ± 0.05</td>
</tr>
</tbody>
</table>

x and y are the number of repeat units in the PEG and PCL blocks, respectively, for each arm of [MePEG$_x$-b-PCL$_y$]$_6$.

$W_{\text{PEG}}$ and $W_{\text{PCL}}$ are the number of water molecules within 3.5 Å of the PEG and PCL blocks, respectively. $W_{\text{PCL/PEG}}$ is the number of water molecules that interact with PEG and PCL simultaneously: $W_{\text{PCL/PEG}} = W_{\text{PEG}} + W_{\text{PCL}} - \text{total number of water molecules interacting with the entire star copolymer}$. $\text{SAS}_{\text{PEG}}$ and $\text{SAS}_{\text{PCL}}$ are the total solvent accessed surface area of PEG and PCL, respectively. $A_{\text{totPCL}}$ is the total surface area of PCL. (a) Values in parentheses are number of water molecules per repeat unit. (b) The surface area of PCL protected by PEG calculated based on MD simulation: $A_{\text{protectedPCL}} = A_{\text{totPCL}} - \text{SAS}_{\text{PCL}}$; $f_{\text{protectedPCL}} = A_{\text{protectedPCL}}/A_{\text{totPCL}}$ represents the fraction of the PCL core protected from water.
Figure 4.5: Solvation properties of various star copolymers, obtained by calculating the number of water molecules within 3.5 Å of the polymers. (a) Double-logarithmic scales of total surface area of PCL core assuming zero protection by PEG. (b) Fraction of PCL at the core surface that is protected from water by PEG, $f_{\text{protectedPCL}}$. (c) Hydration of the PEG corona. Data are evaluated from the last 160 ns of the molecular dynamics simulations. The dashed lines are the linear fit functions.
A collective analysis of these simulations reveals that the total solvent accessible surface area of the hydrophobic PCL core, calculated by the algorithm of Connolly after excluding the PEG blocks, $A_{\text{totPCL}}$, can be predicted according to Equation 4.4. A linear relationship is obtained between $N_{\text{PCL}}$ and $A_{\text{totPCL}}$, as shown in Figure 4.5a.

$$A_{\text{totPCL}} = 364(6N_{\text{PCL}})^{0.627} \text{Å}^2. \quad (R^2 = 0.991) \quad (4.4)$$

As expected, $A_{\text{totPCL}}$ depends only on $N_{\text{PCL}}$, whereas the surface area of PCL that is protected from water by the PEG block, $A_{\text{protectedPCL}}$, is significantly influenced by the PEG blocks such that an increase in $N_{\text{PEG}}$ results in an increase in $A_{\text{protectedPCL}}$ (Table 4.5). Significantly, our results quantify the increase in the fraction of the hydrophobic core protected from water by PEG, $f_{\text{protectedPCL}}$, with an increase in $N_{\text{PEG}}$ (Figure 4.5b), where $f_{\text{protectedPCL}}$ is the ratio of $A_{\text{protectedPCL}}$ to $A_{\text{totPCL}}$:

$$f_{\text{protectedPCL}} = 0.228 \log(6N_{\text{PEG}}) + 0.245. \quad (R^2 = 0.933) \quad (4.5)$$

**Hydration of PEG:** As shown in Table 4.5, large hydration values for PEG blocks, $W_{\text{PEG}}$, are obtained for all SCPs, demonstrating a high solubility for PEG in water. Furthermore, the average hydration number of PEG, $W_{\text{PEG}}$, increases linearly with an increase in $N_{\text{PEG}}$ (Table 4.5 and Figure 4.5c). This yields a theoretical description of $W_{\text{PEG}}$ that can be expressed as:

$$W_{\text{PEG}} = 3.3(6N_{\text{PEG}}) + 74 \text{ (molecules).} \quad (R^2 = 0.996) \quad (4.6)$$

For the various SCPs in this study, the average number of water molecules predicted to bind per PEG repeat unit, $W_{\text{perPEG}}$, ranges from 3.3 to 4.9 (Table 4.5). As well, the average $W_{\text{perPEG}}$ is independent of the PCL block length for all SCPs (Equation 4.6 and Figure 4.5c).
4.4. Discussion

4.4.1. Validation of simulation parameters

The current MD simulations reproduce the known aqueous properties of PCL-\(b\)-PEG blocks copolymers,\(^{269,270}\) wherein the hydrophobic PCL core is largely dehydrated and solvated by a water soluble PEG corona (Figure 4.3a). These fundamental properties emerge from the simulations in spite of the fact that the force fields that are employed do not contain any special terms that dictate solubility explicitly. Rather, hydrophobic collapse in these simulations is driven by the same forces that exist in a test tube, namely the entropically disfavoured formation of an aqueous solvation shell around a non-polar solute. This is consistent with the success of all-atom force fields in computing the self-organization of other micelle- forming molecules, such as detergents,\(^{271}\) as well as the formation of lipid bilayers.\(^{272}\)

Structure and dehydration of the PCL core: The aggregated structure of the PCL core that emerges from MD simulations agrees well with the known properties of this polymer (e.g. water insoluble), confirming that our simulations are sampling the relevant phase of this polymer. In particular, the PCL blocks form a compact hydrophobic core that excludes water and PEG (Figure 4.3a).

The linear relationship between \(R_{g,\text{core}}\) and \(N_{\text{PCL}}\) for all simulated systems (Equation 4.1) indicates a similar packing pattern and architecture for PCL within the hydrophobic cores of all SCPs. For \(N_{\text{PCL}} \geq 4\), the partial overlap of the radial density of PCL and water that is apparent in Figure 4.3b is not the result of actual coexistence. Rather it is an artifact of the radial averaging of an inherently aspherical hydrophobic PCL core (Table 4.4). The same is true of PCL and PEG, which do not mix, beyond the
close interactions of two rough interfaces. For $N_{PCL} \leq 3$, however, the exclusion of water from the PCL core is incomplete. Many studies have proposed that the presence of water at the PCL-PEG interface of micelles promotes hydrolytic degradation of the core, for example, by ester bond cleavage within the PCL blocks. Further, an increase in the rate of degradation of PCL with decreasing MW has been reported based on dynamic light scattering, nuclear magnetic resonance and gel permeation chromatography measurements for $\text{MePEG}_x$-$b$-$\text{PCL}_y$ ($x = 117, y = 7, 13$ and $17$) linear diblock copolymer micelles and $3$-arm ($\text{PEG}_{136}$-$b$-$\text{PCL}_y$)$_3$ SCPs micelles ($y = 11, 18$ and $30$). In the current study, therefore, the presence of water in the interior of the PCL core for $N_{PCL} \leq 3$ suggests that SCPs with $N_{PCL} \leq 3$ may degrade more quickly than SCPs with larger $N_{PCL}$.

**Hydration of the PEG corona:** The present study provides a detailed measurement of polymer hydration at the atomistic level with systematic exploration of polymer MW and conformation (Table 4.5 and Figure 4.5c). Equation 4.6 allows prediction of the hydration of PEG of a known MW within the range of $0.3$ kDa to $5.2$ kDa, which encompasses the PEG polymers employed for numerous biomedical applications. These results are consistent with the known high solubility of PEG in water (Figure 4.5c), which has been suggested to be due to hydrogen bonding between the ether oxygen of the polymer and water. Furthermore, for SCPs with long PEG chains there is an increased probability that water molecules may form a hydrogen bonding “bridge” between PEG arms or PEG repeat units (Appendix B). Such water bridges have been found previously using $^1$H and $^2$H nuclear magnetic resonance measurements for $6$ kDa PEG ($N_{PEG} = 136$) in low water content. In the current study, the decrease in $W_{\text{perPEG}}$ values that occurs with an increase in $N_{PEG}$ from $6$ ($W_{\text{perPEG}} = 4.9$)
to 118 (W$_{perPEG} = 3.5$) is indicative of the replacement of the water-PEG interactions by PEG-water-PEG and/or PEG-PEG interactions (Table 4.5).

As shown in Table 4.5, the average W$_{perPEG}$ obtained from the current study decreased from 4.9 to 3.3 when the N$_{PEG}$ increased from 6 to 118. In comparison, experimental evaluation of PEG of varying MW has yielded estimates of W$_{perPEG}$, with values ranging from 1.0 to 5.0 for linear PEG chains with N$_{PEG}$ in a range of 4.5 to 182.\textsuperscript{28-30} Indeed the estimates obtained experimentally for W$_{perPEG}$ for PEG of similar molecular weights have been quite variable.\textsuperscript{28-31} For example, Branca \textit{et al.} reported a linear relationship between the degree of hydration of linear PEG and MW$_{PEG}$ with W$_{perPEG}$ values increasing from 2.2 to 5.0 when N$_{PEG}$ increased from 4.5 to 45, based on viscosity measurements.\textsuperscript{31} Ng. \textit{et al.}, found the minimum W$_{perPEG}$ to be 2.5, as determined by measuring the heat capacity for the interaction between water and 8 kDa PEG (N$_{PEG} = 182$).\textsuperscript{28} A nuclear magnetic resonance spectroscopy study of 6 kDa PEG (N$_{PEG} = 136$) by Lusse and Arnold suggested that there is a maximum of one water molecule in contact with a single PEG monomer unit.\textsuperscript{29}

As reviewed by Allen \textit{et al.},\textsuperscript{32} the variation in the number of water molecules reported to interact with a single PEG repeat unit may be attributed to differences in PEG conformation which depends largely on MW$_{PEG}$, the architecture of the polymer and the environment (e.g. temperature, concentration, surface tethering, surface density). In this regard, Tirosh \textit{et al.} proposed that the conformations of 2 kDa PEG grafted to liposomes and free in solution are brush (W$_{perPEG} \sim 4.6$) and random coil (W$_{perPEG} \sim 3.0$), respectively.\textsuperscript{30} In another study, it was found that the degree of water uptake depends on the architecture of the SCP such that increasing the number of arms results in a decrease
in hydration as determined by gravimetric analysis of copolymer films. Furthermore, the instruments used to measure the hydration of PEG can affect the hydration results. For example, the $W_{\text{perPEG}}$ values ($N_{\text{PEG}} \sim 4.5$ to 45) obtained by acoustic measurements (1.6 to 2.3) are smaller than those determined by viscosity measurements (2.2 to 5.0). Importantly, the $W_{\text{perPEG}}$ values obtained from the current study directly quantify the number of water molecules that are in contact with the PEG blocks.

### 4.4.2. Relationship to other theoretical and simulation models

Previously, Flory put forth the “random flight” model to predict the size of linear polymers in terms of the $R_g$. In the current study, the size of the PEG chain can be expressed in terms of the $R_g$ based on Flory’s theory, $R_g^{\text{FloryPEG}} = a/\sqrt[6]{N_{\text{PEG}}}^\alpha$ (Appendix B) where $a$ is the size of one PEG repeat unit ($a = 2.8 \text{ Å}$), and $\alpha$ is a unitless coefficient describing the compatibility of the polymer and solvent. As shown in Figure 4.4a, the linear fits from the plot of $MW_{\text{PEG}}$ as function of $R_g^{\text{PEGarm}}$ on a double logarithmic scale yield an $\alpha$ value of 0.479 for the PEG blocks in water (Equation 4.2). This result is similar to the $\alpha$ value for a theta solvent ($\alpha = 0.5$) in which the polymer is said to behave ideally and exist in a Gaussian coil. However, the $\alpha$ value obtained for the PEG blocks from the current study was smaller than the experimental $\alpha$ values obtained for linear PEG in water with $0.2 \text{ kDa} < MW_{\text{PEG}} < 7.5 \text{ kDa}$ ($\alpha = 0.523$) and for single-chain poly(ethylene oxide) (PEO) in water with $25 \text{ kDa} < MW_{\text{PEO}} < 100 \text{ kDa}$ ($\alpha = 0.583$). Furthermore, the current study found that the PEG blocks of the SCPs absorbed to the surface of the PCL core. As can be calculated from data in Table 4.4, the percent difference between the radii of gyration calculated based on Flory theory, $R_g^{\text{FloryPEGarm}}$
(Appendix B), and $R_{g\text{PEGarm}}$ obtained from the current study decreases from 23% to 5% as $N_{\text{PEG}}$ increases from 6 to 118. Recently, a spherical micelle formed from PEG$_{11}$-b-poly($\gamma$-benzyl L-glutamate)$_9$ linear diblock copolymer was studied by all-atom MD for a total simulation time of 7 ns. In order to achieve longer simulation times, Lee et al. parameterized linear PEG into the MARTINI coarse-grained (CG) force field based on all-atom simulation of linear PEG with $9 \leq N_{\text{PEG}} \leq 37$. From these studies, radii of gyration of PEG comparable with experimental values were obtained having $\alpha$ values of 0.51 and 0.515 for $9 \leq N_{\text{PEG}} \leq 36$, and an $\alpha$ value of 0.57 for $36 < N_{\text{PEG}} \leq 158$. In a follow up study based on the MARTINI parameterization of linear PEG, CG simulations of dendrimers composed of PEG-polyamidoamine in water reveal that the PEG blocks (0.55 to 5 kDa) of a densely grafted dendrimer extend outward from the core and stabilize nanoparticles preventing their aggregation. In these studies, the authors noted that the spatial extension of the PEG corona, brush on a hydrophobic surface, agreed with experimental measurement and with theoretical predictions from de Gennes theory. Nevertheless, it should be noted that CG parameters are context dependent. It is unclear if CG parameters developed from atomistic simulations of PEG in water are applicable to the simulation of MePEG$_x$-b-PCL$_y$ in the context of a SCP. Importantly, our results discern the hydrophobic behavior of the SCPs in water and can be employed to parameterize MePEG$_x$-b-PCL$_y$ SCPs into CG models.

In comparison to theoretical and experimental studies that have examined the radius of gyration of PEG, the $\alpha$ value (0.479) obtained from our simulations is smaller indicating that the PEG blocks are more compact in the context of SCPs. This discrepancy may be attributed to the difference in MW of the PEG blocks.
relative to the PCL core and architecture of the copolymer. The conformation of polymers grafted to a curved surface have theoretically been predicted to be dependent on the chain length of the polymer and the radius of curvature. Specifically, when the ratio of the radius of curvature and the thickness of the grafted polymer layer $< 1$, the $R_g$ of the grafted polymers grows as a function of the degree of polymerization of the polymer with $\alpha$ equal to $3/5$. Further, the theoretical model developed by Daoud and Cotton showed that the radius of a star shaped polymer is smaller than that of a linear polymer of the same MW.

4.4.3. Quantification of hydration of the PCL core as a predictor for multimolecular aggregation

Although considerable effort has been expended both theoretically and experimentally to describe the effect of hydration on the macromolecular structure and stability of MePEG-$b$-PCL micelles formed by SCPs or linear diblock copolymers, quantification of the number of water molecules that interact with PCL has remained elusive. For the first time we present a detailed molecular model quantifying the number of water molecules that interacts with the hydrophobic PCL core of various six-arm SCPs. Based on our analysis, the solvent accessible surface area of the hydrophobic PCL core depends not only on the surface area and aggregated structure of the copolymer, but also on the length and conformation of the PEG blocks, whereby increasing PEG block length reduces the exposure of the PCL core to the aqueous environment (Table 4.5 and Figure 4.5b). The $W_{PCL}$ values presented in Table 4.5, however, demonstrate that none of the SCPs in this study contain a PEG corona that is
capable of providing full coverage of the hydrophobic core, exposing segments of the PCL core to water.

In a concentrated aqueous solution of SCPs, the unshielded PCL units would also be in potential contact with PCL units of another unimer, a potential interaction that is highly relevant to self-aggregation. Nevertheless, it is unknown whether the formation of multimolecular micelles is due in part to core-core interaction and/or entanglement of PEG chains of different SCPs. Investigation of aggregation of multiple SCPs is currently in progress in order to better understand the interactions that drive this process. We postulate that the propensity for aggregation of SCPs is correlated with the fractional hydration of the hydrophobic PCL core in the unimolecular state, which is found to vary from 46 % to 23 % for the SCPs investigated in this study depending on the MW of the PCL and PEG blocks (Table 4.5, Figure 4.5b). Sufficient water exposed surface area of the non-polar PCL blocks may destabilize the unimolecular state and thus drive intermolecular association between SCPs resulting in the formation of multimolecular micelles in aqueous media. Accordingly, multimolecular micelles of SCPs with a MW similar to [MePEG\(_{113}\)-b-PCL\(_{18}\)]\(_6\) are observed by dynamic light scattering and transmission electron microscopy at a copolymer concentration of 3.4x10\(^{-6}\) mol/L in water (F. Li and C. Allen, unpublished results). Significantly, the results of the present study are highly quantitative and, as such, may be utilized to rationally design a SCP with a hydrophobic core that is sufficiently protected from water such that it may exclusively form stable unimolecular micelles.
4.4.4. Rational Design of unimolecular micelles

Aggregation between SCPs is likely to result from interactions between water-exposed-PCL segments. The quantitative results of the present study predict the optimal ratio(s) of $N_{\text{PCL}}$ to $N_{\text{PEG}}$ for a SCP in order to minimize the hydration of the PCL core. In particular, extrapolation of Equation 4.5 for maximum protection of the PCL core ($f_{\text{protectedPCL}} = 1$) dictates the minimum $N_{\text{PEG}}$ ($N_{\text{PEG}} = 336$, MW = 14.8 kDa) that is required to completely cover the PCL core. Interestingly, the requisite $N_{\text{PEG}}$ is independent of $N_{\text{PCL}}$ within the confidence interval ($N_{\text{PCL}} \leq 18; 4.2 \leq N_{\text{PEG}} / N_{\text{PCL}} \leq 19.7$, Table 4.1). The hypothetical SCP $[\text{MePEG}_{336}-b-\text{PCL}_{18}]_6$ has a PCL core that we predict is fully protected from solvent and has a predicted $R_{g\text{core}}$ and $R_{g\text{PEGarm}}$ of 13.3 Å and 31.3 Å obtained by utilizing Equations 4.1 and 4.2, respectively. Indeed, PEG blocks of this MW have been employed to stabilize diblock copolymer micelle formulations.276, 284 Importantly, the stability predicted by Equation 4.5 is thermodynamic, distinct from any kinetic stability285,286 that may be afforded by a large PEG corona.

It is important to emphasize that much of the information gained from the MD simulations cannot be obtained by performing experimental studies on the individual SCPs examined in the present study. Experimentally, these SCPs are likely to aggregate in solution, whereas our simulations sample the conformations adopted by unimers at infinite dilution. This allows us to fully characterize the unimolecular state and not only identify the source of thermodynamic instability, but also to quantify the source of instability and thereby predict stable unimolecular compositions. The significance of the present study is the elucidation of quantitative relationships that are instrumental in the rational design of SCPs. The size of the SCP is more strongly related to $N_{\text{PEG}}$ than to
NPCL (Table 4.4), and it is, in many cases, undesirable to increase the size of the unimolecular micelle ad infinitum in order to achieve complete protection of the hydrophobic PCL core from solvent. The quantitative results of this study indicate the minimal size at which the hydrophobic PCL core can be completely protected from water.

4.4.5. Alternative SCP architectures

It remains to be experimentally confirmed whether PEG can completely protect the hydrophobic PCL core of six-armed diblock star copolymers, since the water molecules that interact with PEG at the PEG-PCL interface can also interact with PCL. Regardless of PEG length, there is either a small amount of water interacting with the hydrophobic core or a small amount of proximal PEG that is unfavourably dehydrated. Inclusion of an amphiphilic block between the hydrophobic and hydrophilic blocks may be desirable in some cases.

Furthermore, the architecture of the SCP may need to be modified in combination with optimization of the ratio of the PEG and PCL block lengths. As well, the chemical structure of the central connector may need to be considered in order to utilize the relationship between the structural properties and MW of the PCL core for other SCPs that have chemically different connectors. Recently, Schramm et al. synthesized many SCPs having 4 and 6 arm PCL₁₂ cores (N_{PCL} = 12) that are conjugated to 4-, 6-, 8- and 12-branched-PEG (N_{PEG} = 8 to 30) per PCL arm. Interestingly, these SCPs behave as unimolecular micelles according to dynamic light scattering measurements. Nevertheless, simply increasing the number of arms of the SCP does not necessarily result in a
unimolecular micelle. For example, a previous experimental study revealed that (MePEG$_{113}$-b-PCL$_{26}$)$_{16}$ SCPs formed multimolecular micelles (CMC of ca. 3 mg/mL).$^{249}$

4.5. Conclusions

Our simulations elucidate the solution behavior of (MePEG$_{x}$-b-PCL$_{y}$)$_{6}$ SCPs in water and predict a densely packed hydrophobic core that is phase separated from a highly mobile hydrophilic PEG corona. The average number of water molecules predicted to bind per PEG repeat unit is in the range of 3.3 to 4.9, in good agreement with experimental data for SCPs with high MW$_{PEG}$. The conformation of the hydrophobic PCL core and the conformational and solution properties of the PEG shell are predictable and independent of the ratio of hydrophilic to hydrophobic block length. This report reveals that, with the PCL and PEG composition investigated in this study, the PEG corona of six-armed diblock SCPs provides only partial coverage of the hydrophobic core, leaving segments of PCL exposed to water. Although it is likely that a simple increase in the amount of PEG will be sufficient to entirely encapsulate the PCL core and protect the SCPs from PCL mediated aggregation, the MW of the SCP also needs to be sufficiently small for utilization of the material for a particular application (e.g. drug delivery). Unique to this study, the hydration of the PCL core is quantified as a function of $N_{PCL}$ and $N_{PEG}$, enabling prediction of the minimum $N_{PEG}$ required for complete protection of a core with a specific MW$_{PCL}$. Overall, this study provides fundamental insight into the properties of star copolymers in aqueous solution that should be useful for rationally designing a second generation of star copolymers that exclusively form unimolecular micelles. In addition, the systematic methodology developed in this study is applicable to
the design of arbitrarily composed polymers for which the principle requirement is the protection of a hydrophobic surface from aqueous solution.

4.6. Acknowledgments

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Chapter 5: Current and Future Directions

5.1. Introduction

The investigation of drug enhancement has been brought to its logical conclusion in Chapters 2 and 3 of this thesis: synthesis of a more soluble DTX prodrug that is hydrolyzed to release active drug under biologically relevant conditions.

The results obtained from simulation studies of [MePEG$_x$-b-PCL$_y$]$_6$ SCPs suggest a number of follow-up simulations. These additional simulations will further our understanding of other important properties that control the potential of utilizing the [MePEG$_x$-b-PCL$_y$]$_6$ SCPs as a material for drug delivery. They will also directly probe the mechanism of aggregation of the SCPs. These studies will provide insight into the following areas:

1. The partitioning of small hydrophobic molecules between aqueous solution and SCPs of various molecular weights.
2. The aggregation mechanism of SCPs in a concentrated aqueous solution.
3. The drug loading and aggregation properties of various branching architectures of SCPs.

5.2. Partitioning of Small Hydrophobic Molecules in Aqueous Solution of SCPs.

Drug delivery materials must be capable of loading, protecting, transporting, and releasing drug molecules at the target tissue. Stable nanoparticle delivery systems that retain their cargo in vivo until reaching the target site and release the active agent once at the desired site can result in significant improvements in the therapeutic index of a drug and reduce undesired side effects that result from the distribution of drug to non-target
tissues.\textsuperscript{11,12} Often, architectural features of linear block copolymer micelles, including the nature and MW of hydrophobic/hydrophilic blocks, can affect the performance of the micelles in terms of drug loading and release. However, little is known about the thermodynamics and kinetics of drug release as well as the drug loading capacity of unimolecular SCP micelles. As such, understanding the relationship between the architecture of these materials and their interaction with lipophilic compounds is necessary for their use in particular applications, such as the delivery of lipophilic agents.

In this context, I am currently evaluating the dynamics and partitioning of a hydrophobic drug mimetic in the presence of SCP micelles. A series of atomistic simulations consider variations in the MWs of the hydrophobic and hydrophilic blocks. These studies will reveal important properties such as drug loading levels, drug retention time, degree of drug exposure to water within the formulation, and molecular mechanism of drug release.

At present, there are five simulated systems, each containing a single hydrated [MePEG\textsubscript{x}-\textit{b}-PCL\textsubscript{y}]\textsubscript{6} SCP and a variable number of benzene molecules. In this study, benzene was chosen as a drug mimetic because simulations using available benzene force field parameters reproduce known quantities. Benzene is also small and highly symmetric, and thus simulation convergence will be attained more quickly. The SCPs vary systematically in terms of the degree of polymerization of the PEG and PCL in each arm (\textit{i.e.} number of repeat units, \textit{x} and \textit{y}, respectively) as well as in terms of total MW of the SCP. The composition and size of each system used in the MD simulations is outlined in Table 5.1.
Table 5.1: Composition of the \([\text{MePEG}_{x-b}\text{-PCL}_y]_6\) star copolymer in the presence of benzene molecules in the molecular dynamics simulations.

<table>
<thead>
<tr>
<th>(x-y)/B</th>
<th>Water (molecules)</th>
<th>MW of PCL:PEG per arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>[27-4]/30</td>
<td>17222</td>
<td>456:560</td>
</tr>
<tr>
<td>[38-4]/30</td>
<td>16006</td>
<td>456:868</td>
</tr>
<tr>
<td>[38-9]/67</td>
<td>15090</td>
<td>1026:868</td>
</tr>
<tr>
<td>[118-6]/102</td>
<td>108123</td>
<td>684:5230</td>
</tr>
<tr>
<td>[113-18]/102</td>
<td>107754</td>
<td>2052:5010</td>
</tr>
</tbody>
</table>

x and y are the number of repeat units in the PEG and PCL blocks, respectively, of each arm in the \([\text{MePEG}_{x-b}\text{-PCL}_y]_6\) star copolymers. B is the number of benzene molecules included in the system.
During the MD simulation, in the presence of benzene, the PCL blocks maintain a compact core that excludes water, while the hydrophilic PEG blocks remain highly hydrated, as shown in Figure 5.1. As predicted, benzene molecules partition more favourably into the PCL core than water. Importantly, statistical sampling convergence is attained because the simulations are long enough to achieve steady-state equilibrium, with equal average numbers of benzene molecules diffusing in and out of the PCL core.

**Figure 5.1:** Density profile of PCL core (♦), PEG blocks (▲), benzene (♦), water (ο) and whole system (◊) as a function of distance from the center of mass of the PCL core averaged at (a) time = 1 ns and (b) over the last 85 ns of simulation of [MePEG$_{113}$-b-PCL$_{18}$]$_6$ in the presence of benzene.
Overall, benzene molecules within the SCP can be found mainly in three regions including the inner PCL core region, the PEG/PCL interface region, and the PEG shell region (Figure 5.2). Within the inner core region, benzene molecules are solvated by the PCL core and are completely dehydrated as shown in Figure 5.2. Within the PEG/PCL interface region, benzene molecules are solvated by the PCL blocks and may or may not interact with water molecules, although if interactions are present, they are always very few. These benzene molecules have a shorter average residence time, i.e. faster release rate, in comparison to benzene molecules in the inner PCL core region. When the inner PCL core and the PEG/PCL interface regions are saturated with benzene molecules, benzene molecules reside also in the PEG shell.
Figure 5.2: (a) Fraction of hydrated (◊), intermediate (▲) and completely dehydrated (■) states of benzene molecules in [MePEG\textsubscript{113}-b-PCL\textsubscript{18}]\textsubscript{6} SCP as a function of time. The interaction of benzene with water and star copolymer of the simulated system is converged after 60 ns. (b) Snapshot of the benzene molecules (green) in the inner core PCL (yellow), at interface of PCL and PEG (blue) and in bulk water (red). Water molecules within 4 Å of benzene are shown explicitly and calculated to quantified the dehydrated (zero water molecule), intermediate (0 < water molecules \leq 2) and completely hydrated (>19 water molecules) states. Boundary between hydrated states were place at local minima on a plot of probability of hydration of benzene in various [MePEG\textsubscript{x}-b-PCL\textsubscript{y}]\textsubscript{6} SCPs. Benzene molecules with hydration number between 3 and 19 will be quantified in the future and are not shown for clarity. Bulk water and hydrogen atoms of the PCL and PEG blocks are omitted for clarity.
The size of the PCL core in the presence of benzene is slightly greater than in the absence of benzene. These results may be helpful in determining the optimal drug load for a particular MW of PCL. Further, preliminary results from this study suggest that high-MW PEG can slow the release of benzene from a hydrophobic core in comparison to that of a SCP with lower MW PEG.

In terms of drug loading and release, preliminary results from our study revealed that high MW\textsubscript{PEG} can slow the release of drug from a hydrophobic core in comparison to that of a SCP with lower MW\textsubscript{PEG} (Figure 5.3). The preliminary kinetics of benzene molecules binding to the PCL core is summarized in Table 5.2. Additional quantities to be measured include the binding energy, the partitioning coefficient of benzene molecules into the PCL core, and the relationship between the size of the PCL core and the number of benzene molecules entrapped by the core.
Figure 5.3: Probability of benzene molecules interact with the PCL core during MD simulations. \( \tau \) is the average lifetime of benzene molecules interacting with the PCL core. \( t_{1/2} \) is the half life, \( t_{1/2} = \tau \ln 2 \)

Table 5.2: Binding kinetics of benzene molecules to the \([\text{MePEG}_x-b\text{-PCL}_y]_6\) star copolymer.

<table>
<thead>
<tr>
<th>[x-y]/B</th>
<th>( \tau ) (ns)</th>
<th>( t_{1/2} ) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[27-4]/30</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>[38-4]/30</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>[38-9]/67</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>[118-6]/102</td>
<td>8.6</td>
<td>5.9</td>
</tr>
<tr>
<td>[113-18]/102</td>
<td>7.5</td>
<td>5.2</td>
</tr>
</tbody>
</table>

\( \tau \) is the average lifetime of benzene molecules interacting with the PCL core. 
\( t_{1/2} \) is the half life, \( t_{1/2} = \tau \ln 2 \).
Overall, this study suggests that better drug loading and retention can be obtained with higher MW of PCL and PEG blocks, respectively. Further quantification will be performed including the hydration of the PCL core and PEG shell in the presence of benzene molecules. This information may be helpful in predicting the impact of small hydrophobic molecules on the surface area of PCL exposed to water, and thus on the tendency of SCPs to aggregate. Additional quantities related to drug retention and loading to be measured include the partition coefficient of benzene molecules between the PCL core and water as a function of the size of the PCL core. In the future, the potential use of SCPs as drug delivery vehicles can be further investigated by simulating the partitioning of benzene and other drug mimetics into the SCP that was predicted to form unimolecular micelles in water in Chapter 4 ([MePEG336-b-PCL18]6 SCP).

5.3. Aggregation Mechanism of Multiple Star Copolymers

The experimental study of [MePEG113-b-PCL18]₆ SCPs indicated that these SCPs aggregated and formed a multimolecular micelle. However, experimental determination of the aggregation mechanism can be very difficult. Previously, I predicted that PCL core-core interaction of different SCPs is one of the potential causes of aggregation of the SCPs (Chapter 4). However, it is unknown if formation of multimolecular micelles could also be due to other physical interactions such as the entanglement of the PEG chains from different SCPs. Therefore, elucidate the molecular mechanism of PCL self-aggregation, simulations of systems containing two or more [MePEGₙ-b-PCLₘ]₆ SCPs with various PEG and PCL lengths (e.g. x = 38, 113, 336, and y = 6, 9, 18) can be
performed in the future. Atomistic simulation of these large systems can capture the aggregation features in atomistic detail.

Nowadays, advanced simulation techniques and the availability of powerful computers allow the simulations of large systems with millions of atoms extensively to the microsecond timescale. Atomistic MD simulations of hydrated multimolecular \([\text{MePEG}_{38-b}-\text{PCL}_{6}]_{6}\), \([\text{MePEG}_{38-b}-\text{PCL}_{9}]_{6}\), \([\text{MePEG}_{113-b}-\text{PCL}_{18}]_{6}\) SCPs systems are currently in progress in order to investigate the aggregated structure and interactions that drive the formation of multimolecular SCPs micelles. These systems contain up to one million atoms with a cumulative simulation time currently approaching one microsecond. Some preliminary results from these simulations are presented below.

Snapshots of the conformation of eight \([\text{MePEG}_{38-b}-\text{PCL}_{6}]_{6}\) SCPs and eight \([\text{MePEG}_{113-b}-\text{PCL}_{18}]_{6}\) SCPs following 1000 ns and 650 ns of simulation, respectively, are shown in Figure 5.4. In agreement with the predictions from the simulation of various single SCPs in water, preliminary results indicate that the interactions between these SCPs involve core-core interactions as shown in Figure 5.4. Therefore, future studies will investigate the impact of the intermolecular core-core interactions on the loading of drug mimetics as well as the impact of dilution on the release of encapsulated drug. Furthermore, the relative drug affinities of unimeric and multimolecular SCPs will be computed.
Figure 5.4: Snapshot of molecular simulation of (a) eight [MePEG\textsubscript{38}-\textit{b}-PCL\textsubscript{6}]\textsubscript{6} and (b) eight [MePEG\textsubscript{113}-\textit{b}-PCL\textsubscript{18}]\textsubscript{6} SCPs in water at time $t = 1000$ ns and $t = 650$ ns, respectively. The PEG blocks are represented in blue lines, and PCL cores are represented as multicoloured balls. The water molecules are omitted for clarity.
In the future, statistical sampling convergence of these simulation will be examined. Further, other quantitative measurements based on MD simulation of the multiple SCPs systems will be performed in order to investigate other important properties that are related to the aggregation morphology and drug loading capacity of these SCPs.

Alternatively, coarse grained models can be developed and used in order to speed up the simulation of multiple SCPs. Importantly, the atomistic parameters developed for SCPs and the lengthy systematic simulation in the above study provide all the information necessary to parameterize SCPs into coarse grained models.

5.4 Alternative SCP Architectures

The development of unimolecular SCP micelles for drug delivery has been extensively investigated by many groups.\textsuperscript{61, 137, 140, 251, 252, 287} In only a few cases, however, has the formation of unimolecular micelles been reported.\textsuperscript{137} One study has shown that simply increasing the number of arms of the SCP without optimizing the ratio of the PEG and PCL block lengths, \textit{e.g.} $[\text{MePEG}_{113}-b-\text{PCL}_{26}]_{16}$ SCPs, does not necessarily result in a unimolecular micelle.\textsuperscript{249} In another experimental study, a unimeric SCP micelle was obtained by increasing the number of PEG branches while keeping the number of PCL branches constant, such as SCPs having 4 and 6 arm PCL that are conjugated to 4-, 6-, 8- and 12-branched-PEG per PCL arm.\textsuperscript{137} The results from these studies suggest that new architectures of the SCP in combination with optimization of the ratio of the PEG and PCL block lengths may be required in order to obtain unimolecular micelles.
One of the significant aspects of the studies presented in this thesis is that the systematic methodology developed for the investigation of [MePEG$_x$-b-PCL$_y$]$_6$ SCPs is applicable to materials with different architectures. Therefore, this method can be used to rationally design different architectures of SCPs that have the potential of forming unimolecular micelles in water. In particular, future MD simulations can be carried out for SCPs in which the number of PEG branches is increased while keeping the number of PCL branches constant, e.g. [n(MePEG$_x$)-b-PCL$_y$]$_6$ SCPs with n = 2, 4, 6, etc. Further, the degree of polymerization of the PEG and PCL blocks can be varied in order to optimize the PEG:PCL ratio. Alternatively, different chemical linkers between the hydrophobic and hydrophilic blocks may be investigated in order to minimize the water interacting with the hydrophobic core at the hydrophobic/hydrophilic interface. These studies may provide a useful library of SCPs for the development of drug delivery materials.
Chapter 6: Conclusions

Developing drug formulations is often very difficult, especially for molecules possessing poor aqueous solubility. The development process is also very time consuming because numerous variables that must be optimized in order to obtain a formulation with the desired properties. To reduce investigation time and cost, theoretical approaches can be employed to reduce or eliminate the experimental trial and error approach to drug formulation and fast track development efforts. In this thesis, I have applied two main strategies to develop new formulations with better performance. First, I have examined factors that influence the compatibility of a drug and formulation material. Based on these results, I have designed and synthesized prodrugs that are more compatible with the delivery material. Second, I have investigated the fundamental properties of a unique delivery system at the atomistic level and proposed a design with optimal composition so that it can form stable unimolecular micelles for the solubilization of hydrophobic compounds. The work presented in this thesis contributed to three peer-reviewed articles 40, 48, 49 which form the basic of Chapters 2 to 4 and summarized below.

First, I investigated the solubility of a drug in various formulation materials using a combination of theoretical and experimental approaches, as presented in Chapter 2. In this work, I studied the hydrophobic anti-cancer agent docetaxel (DTX) formulated an oil-in-water nano-emulsion. This nano-emulsion comprises an oily inner phase and a hydrophilic outer phase. The oily phase forms the hydrophobic inner core for encapsulation of the hydrophobic drug. This hydrophobic core is protected and solubilized by hydrophilic surfactants that form the outer shell of the nano-emulsion.
This work mainly focuses on investigating the compatibility between the drug and oily excipients (i.e. triglycerides, vitamin E and β-caryophyllene) that form the hydrophobic core of the nano-emulsion. To this end, I employed semi-empirical calculations and MD simulation methods. In this study, I determined that semi-empirical methods are suitable for estimating solubility parameter values and for screening materials that are similar in structure. Additionally, the MD method provided accurate estimates of the solubilities of DTX in various materials. In particular, the MD-based predictions of the solubility of DTX in various excipients were within 2 to 6 % of the experimental values. This work demonstrated that these computational models are reliable analytical tools for selecting suitable formulation components while developing drug formulations.

To enhance the speed of computation, the above study was limited to evaluating drug solubility. However, high levels of drug loading and retention in the formulation are also requirements of an optimal drug formulation. Generally, the extent of drug loading and retention can be significantly improved by increasing the compatibility between the drug and the solubilizing media of the delivery system. The results presented in Chapter 2 lead to the hypothesis that the conjugation of DTX to a moiety that is chemically similar to that of the drug solubilizing media can significantly enhance the compatibility between drug and formulation material. In this context, in Chapter 3 consideration of semi-empirical solubility parameters and partition coefficient values was used to rationally design DTX conjugates. The theoretically-identified optimal drug derivatives were then synthesized and evaluated experimentally in terms of drug retention and solubility as well as drug loading in nano-emulsions. As predicted, the experimental solubility and actual drug loading of the DTX conjugates were increased significantly in comparison to DTX.
These enhancements were attributed to the increase in the hydrophobic compatibility between the drug conjugates and the emulsion. Furthermore, one of the drug derivatives is hydrolyzable under biologically-relevant conditions to yield an active drug. Overall, this study demonstrates that conjugation of DTX to a moiety, lauroyl fatty acid, that resembles the chemical structure of the solubilizing media, triglycerides, is a promising strategy for enhancing the drug loading and encapsulation efficiency in a formulation.

Whereas the studies presented in Chapters 2 and 3 focus on developing drugs for formulations, the third study, presented in Chapter 4, focuses on developing a material for drug delivery. In particular, I investigated the thermodynamic properties of \([\text{MePEG}_x-b-\text{PCL}_y]\)_6 SCP micelles including conformation, size and the solvation properties of the hydrophobic core and hydrophilic shell. In principle, SCPs have the potential to form unimeric micelles that are thermodynamically stable at infinite dilution. In practice, an experimental study from our group indicated that the \([\text{MePEG}_{113}-b-\text{PCL}_{18}]_6\) SCP can self-aggregate and form multimolecular micelles, which are inherently unstable to dilution. In order to elucidate the cause of this aggregation, I systematically studied the solution behaviour of these SCPs with different ratios of hydrophobic and hydrophilic block lengths. To this end, atomistic MD simulations were used to investigate unimeric SCPs in water. Such a thing is difficult to do experimentally because of the low concentration required. In this study, the focus is on quantifying the water accessibility of the hydrophobic core, which impacts the tendency of SCPs to form multimolecular micelles. Based on these simulation results, I postulated that aggregation of SCPs is mediated by segments of the hydrophobic core that are exposed to water prior to aggregation. Therefore, I present that it is possible to modify the composition of the SCP to obtain an
optimal ratio of hydrophobic PCL core to hydrophilic PEG corona. I do this by determining and then extrapolating the relationship between the hydration of PCL core and the MW of PEG. Here, the word optimal is emphasized because the requirements that the hydrophilic corona forming material be large enough to cover the hydrophobic PCL core, hence the formation of unimeric SCP micelles, is not the only criterion that must be satisfied. In addition, the hydrophilic corona must also be small enough that the overall size of the unimeric micelle is appropriate for a particular application. This often means that the size should be minimized. My results are keys to determining the appropriate compromise between these two extremes. Overall, the systematic MD simulations of various SCPs in water highlight factors that influence the aggregation of SCP micelles and indirectly probe the mechanism of multimolecular micelle formation.
Appendix A

A.1. Flory-Huggins Theory

The rationale between $\chi_{\text{FH}}$ being greater than 0.5 and phase separation of a binary mixture may be explained by consideration of FH theory.\textsuperscript{45} $\chi_{\text{FH}}$ was originally put forth to describe the miscibility of polymer-solvent mixtures. In more recent years, FH theory has been used to characterize the thermodynamics of polymer-polymer, polymer-drug, solvent-solvent\textsuperscript{203} and other mixtures\textsuperscript{188}. According to FH theory,\textsuperscript{45} the relationship between the $\chi_{\text{FH}}$ and the Gibbs energy of mixing ($\Delta G_{\text{mix}}$) may be described by considering eqs A1-A3. Specifically, the entropy change ($\Delta S_{\text{mix}}$) and enthalpy change ($\Delta H_{\text{mix}}$) upon mixing a polymer and solvent can be calculated using eq A1 and A2, respectively:

$$\Delta S_{\text{mix}} = -k (N_1 \ln \phi_1 + N_2 \ln \phi_2) \quad \text{(A1)}$$

$$\Delta H_{\text{mix}} = k T N_1 \phi_2 \chi_{\text{FH}} \quad \text{(A2)}$$

where $N_i$ and $\phi_i$ are the number of molecules and volume fraction of solvent and polymer, respectively. $k$ is the Boltzmann constant. The $\Delta G_{\text{mix}}$ can then be obtained by combining eqs A1 and A2 as follows:

$$\Delta G_{\text{mix}} = kT [N_1 \ln \phi_1 + N_2 \ln \phi_2 + N_1 \phi_2 \chi_{\text{FH}}] \quad \text{(A3)}$$

By differentiating eq A3 with respect to the number of molecules of solvent $N_1$ and then multiplying the result by Avogadro’s number the partial molar Gibbs energy of mixing is obtained which can be expressed in terms of the chemical potentials of the pure solvent ($\mu_i^0$) and the solvent in the solution ($\mu_i$) as shown in eq A4.\textsuperscript{45, 187, 189}

$$\mu_1 - \mu_1^0 = RT \left[ \ln (1 - \phi_2) + \left( 1 - \frac{1}{x} \right) \phi_2 + \chi_{\text{FH}} \phi_2^2 \right] \quad \text{(A4)}$$
where \( x \) is the number of chain segments of the polymer. As established by Flory,\(^{45}\) the “critical” conditions for the onset of phase separation are:\(^{45, 187, 189}\)

\[
\left(\frac{\partial \mu_1}{\partial \phi_2}\right)_{T, P} = \left(\frac{\partial^2 \mu_1}{\partial \phi_2^2}\right)_{T, P} = 0
\]  

(A5)

Application of the above critical conditions to eq A4 leads to the first (eq A6) and second (eq A7) derivative\(^ {45, 187, 189}\) of eq A4 with respect to volume fraction of polymer:

\[
(1 - \phi_{2, \text{crit}})^{-1} - (1 - 1/x_n) - 2 \phi_{2, \text{crit}} \chi_{FH, \text{crit}} = 0
\]  

(A6)

\[
(1 - \phi_{2, \text{crit}})^{-2} - 2 \chi_{FH, \text{crit}} = 0
\]  

(A7)

\[
\chi_{FH, \text{crit}} = \frac{1}{2} (1 - \phi_{2, \text{crit}})^{-2}
\]  

(A8)

Combination of eqs A6 and A7 yield eq A8 which demonstrates that the \( \chi_{FH, \text{crit}} \) approaches 0.5 as the volume fraction of the polymer approaches zero (\( i.e. \) the minimum value for \( \chi_{FH, \text{crit}} \) is 0.5).

In this study, the FH model was employed to predict the solubility of DTX in various excipients with the assumption that the value for \( \chi_{FH, \text{crit}} \) was equal to 0.5 for the drug-solvent mixture.

A.2. MD Theory

In general, MD is based on Newton’s equation of motion\(^ {183}\):

\[
F_i (t) = m_i \ a_i (t)
\]  

(A9)

where \( F_i \) is the force, \( m_i \) is the atomic mass and \( a_i \) is the acceleration of the atom \( i \). The derivative of the potential energy \( V \) with respect to the coordinates \( r_i \) at time \( t_i \) can be used directly to calculate the force on the atom \( i \).
\[ \frac{\partial V}{\partial r_i} = m_i \frac{\partial^2 r_i}{\partial t_i^2} \] 

At the beginning of an MD simulation, the initial coordinates and velocities are first defined based on the temperature of the system. Then, the Verlet leapfrog integrator \(^{171}\) is used to determine the coordinates and velocities at earlier and later times based on the following equations:

\[ r_i(t+\Delta t) = r_i(t) + \Delta t v_i \left( t + \frac{\Delta t}{2} \right) \] 

\[ a_i(t+\Delta t) = \frac{F_i(t+\Delta t)}{m_i} \] 

\[ v_i(t+\Delta t) = v_i \left( t - \frac{\Delta t}{2} \right) + \Delta t a_i(t) \]

where \( i = 1, ..., N \) and \( N \) is the number of atoms in the system, \( r_i(t) \) is the position, \( v_i(t) \) is the velocity and \( a_i(t) \) is the acceleration at time \( t \) of atom \( i \). Coordinates and velocities for a complete MD simulation are recorded in the trajectory file \(^{171}\).

The total potential energy \( (E_{\text{total}}) \) of a system can be obtained from the coordinates of the system combined with the functional forms and parameter sets of the COMPASS force-field as shown in eq A14 \(^{184, 186}\).
The first eleven functions in eq A14 correspond to the valence and off-diagonal cross-coupling terms. These terms are expressed as functions of bond length (b), bond angle (θ), torsion angle (φ), out-of-plane angle (χ) and the force constants (Ki) determined from the quantum mechanical energy surface. The last two functions in eq A14 represent the non-bond interactions (i.e. Coulombic and van der Waals (vdW) interactions) which are used to calculate the inter- and intra-molecular interaction energy between pairs of atoms that are separated by two or more atoms. The Coulombic term accounts for electrostatic interaction energy between partial charges of the atoms i and j (q) that are separated by a distance rij. The vdW interaction is described by a “soft” Lennard–Jones-9-6 function as expressed in eq A15.
\[ \varepsilon_{ij} = 2 \sqrt{\varepsilon_i \varepsilon_j} \frac{r_i^3 r_j^3}{(r_i^*)^6 + (r_j^*)^6} \]  

(A16)

\[ r_{ij}^* = \left( \frac{(r_i^*)^6 + (r_j^*)^6}{2} \right)^{1/6} \]  

(A17)

where \( \varepsilon_{ij} \) and \( r_{ij}^* \) are constants for a pair of particles \( i \) and \( j \) at a distance \( r_{ij} \).
Appendix B

B.1. Asphericity of Hydrophobic PCL Core

Many studies have been put forth to measure the asphericity of a cluster or group.\textsuperscript{196,288} In this study, the asphericity of the hydrophobic PCL core was determined by employing a theoretical model that was established by Bruns and Carl as shown in Equation B1.\textsuperscript{196}

\[
\text{Asphericity} = 1 - 3 \frac{x^2 y^2 + y^2 z^2 + z^2 x^2}{(x^2 + y^2 + z^2)^2}
\]  \hspace{1cm} (B1)

where \(x\), \(y\) and \(z\) are the principal moments. An asphericity value of 0 is achieved for a perfect sphere. The asphericity of the core causes a broadened distribution of the PCL and PEG blocks as well as water (Figure B1). This broadening results in a significant overlap between the radial density distribution curves, although the PEG blocks do not penetrate into the collapsed hydrophobic PCL core of the SCPs, i.e. the PCL core excludes not only the water, but also the PEG blocks. As shown in Figure B1, the density of PCL (\(N_{\text{PCL}} = 18\)) that is obtained from the MD simulations is similar to the bulk density reported for PCL (1.15 g·cm\(^{-3}\)) of a similar MW.\textsuperscript{289}
Figure B1: Density profiles of PEG (*), PCL (--), water (*) and the entire [MePEGₓ-b-PCLᵧ]₆ star copolymer (□) as a function of distance from the center of mass of the hydrophobic core, averaged over the last 160 ns. The x and y values are labeled at the top right of the plots.
B.2. End-to-End distance and Conformation of PEG

As shown in Figure B2, the EED_{PEGarm} of the PEG blocks of the SCPs investigated in this study increase with increasing MW_{PEG}. For SCPs with N_{PEG} \leq 12, the EED_{PEGarm} distribution of each PEG arm is similar within a given SCP. In contrast, the distribution of EED_{PEGarm} differs amongst arms for SCP with N_{PEG} \geq 19, for which PEG arm sampling remains unconverged within 200 ns (Figure B2). Nevertheless, the radius of gyration of PEG, averaged over all arms converges for all SCPs investigated in this study (Figure B3). For SCPs with N_{PEG} > 12, the distal ends of the PEG blocks often extend away from the center of the hydrophobic core, and then curl back providing protection of the hydrophobic core (see trajectory movie). Furthermore, hydrogen-bond “bridges” between PEG arms or PEG repeat units were observed as shown in Figure B4.
**Figure B2:** The distribution of the end-to-end distance of each of the PEG blocks of all [MePEG$_x$-$b$-PCL$_y$]$_6$ star copolymers, averaged over the last 160 ns of the molecular dynamics simulation. The x and y values are labeled at the top right of the plots.
**Figure B3**: The hydration of PCL core (♦) and PEG blocks (◊) and the radius of gyration of PCL core (▲) and PEG blocks (▼) of [MePEG_x-b-PCL_y]_6 star copolymer as a function of simulation time. The x and y values are labeled at the top right of the plots.
Figure B4: (a) Hydrogen bond (green line) formed between water molecule and the oxygen atoms of (a) PEG repeat units within the same PEG block or (b) “bridging” two PEG repeat units from different PEG blocks. (c) Radial distribution functions between hydrogen atoms of PEG and oxygen atoms of water molecules (▲) oxygen atoms of PEG and hydrogen atom of water molecules (●).
B.3. Theoretical Model for Predicting the Structural Properties of PEG

Previously, Flory put forth the “random flight” model to predict the size of linear polymers in terms of the radius of gyration, Rg (the average root mean square distance of a segment from the COM of the molecule).\(^4\)\(^5\) Rg is approximately proportional to \(R/\sqrt{6}\), where R, the root mean square end-to-end distance, scales with the size of the monomer, a, and the degree of polymerization of the polymer to a power law (i.e. \(R \approx aN^\alpha\)).\(^1\)\(^\text{1}7\) In a theta solvent, the polymer behaves ideally and exists as a Gaussian coil with an \(\alpha\) value of 0.5. In a poor solvent, the polymer is closely packed and has an \(\alpha\) value of \(\frac{1}{3}\).\(^1\)\(^\text{1}7\) In a good solvent, the radius (R) is known as the Flory radius with an \(\alpha\) value of 0.588 (\(R_F = aN^\alpha\)).\(^1\)\(^\text{1}7\) In the current study, the size of the PEG chain can be expressed in terms of the Rg based on Flory’s theory:

\[
R_g^{\text{FloryPEG}} = \frac{a}{\sqrt{6}} N_{\text{PEG}}^\alpha, \quad \text{where} \quad a = 0.28 \text{ nm for PEG.}\(^1\)\(^\text{97}\)
\]  

(B2)
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