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Validation of a Novel Molecular Dynamics Simulation Approach for Lipophilic Drug Incorporation into Polymer Micelles

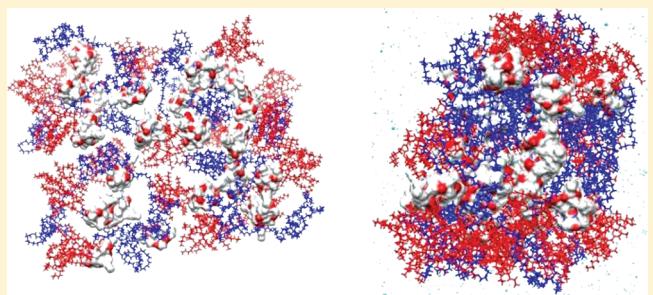
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ABSTRACT: Polymer micelles can be used to facilitate the aqueous solubilization of lipophilic, poorly water-soluble compounds and drugs. Even if the evaluation of the efficiency of drug incorporation into such micelles can be tested experimentally, a theoretical approach based on molecular simulation can constitute a useful tool that reduces time and cost. Here we present a promising method, based on molecular dynamics simulation, for the calculation of the Flory–Huggins interaction parameters as a measure of the potential for drug incorporation into polymer micelles. The data from modeling are validated on four drug compounds with different physical-chemical properties by means of a comparison with the data obtained from experiments.



1. INTRODUCTION

Currently, the most common methods for drug delivery are tablets and aqueous solutions. However, many newly developed drugs possess poor water solubility, which makes it complicated or even impossible to use the above-mentioned pharmaceutical formulations. Devising formulations of many potent but poorly water-soluble drugs with sufficient drug concentrations is a major and challenging problem in pharmaceutical technology. Approaches to solve this problem are to incorporate such lipophilic drugs into nanoparticles, liposomes, emulsions, or polymer micelles to compensate for their poor solubility.¹ Polymer micelles are promising drug carriers since they allow for high drug loadings and owing to typical sizes in the nanometer range.² Such micelles are formed by amphiphilic copolymers having hydrophilic and lipophilic parts, which can be organized into different structures such as linear di- and triblock, graft, random, or star-shaped copolymers.³ Linear diblock copolymers are widely studied for pharmaceutical technology applications.⁴ In aqueous solutions with concentrations lower than the critical micellar concentration (cmc), they exist as single units, and with concentrations that are higher than the cmc, they self-assemble to form polymer micelles. Usually, micelles are built with several dozens of molecules,¹ in which the lipophilic parts form the micelle's core and the hydrophilic parts form the micelle's corona. Important for pharmaceutical formulations is the fact that lipophilic drugs can be incorporated into the lipophilic cores, and such drug-loaded micelles are water-soluble by the hydrophilicity of the corona.

A variety of suitable block copolymers can be used to facilitate lipophilic drug solubilization. Widely studied copolymers are, for example, the diblock copolymers poly(ethylene oxide)-*b*-poly(propylene oxide) (PEO-*b*-PPO) and the triblock copolymers (PEO-*b*-PPO-*b*-PEO) known as Pluronics.⁵ These copolymers are nontoxic and possess a low cmc, which leads to higher micelle stability upon dilution than is achieved with classical small-molecule surfactants.⁶ However, they are non-biodegradable.

Currently, there is notable interest in biocompatible and biodegradable polymers as drug delivery systems. In vivo degradation into units, which can be further easily eliminated from the human body, allows for multiple applications and avoids the need for surgical removal after the application.

A commonly studied biodegradable diblock copolymer is poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) (PEO-*b*-PCL). Several poorly water-soluble drugs have been successfully incorporated into PEO-*b*-PCL micelles, for example, cyclosporin A,⁷ paclitaxel,⁸ or curcumin.⁹ Molavi et al. studied the incorporation of cucurbitacin I and B into PEO-*b*-PCL micelles of different molar masses. The solubility of these drugs increased significantly from 0.05 to 0.30–0.44 and 0.65–0.68 mg/mL, respectively, for PEO₅₀₀₀-PCL_{5000 g/mol} and PEO₅₀₀₀-PCL_{24000 g/mol} diblock copolymers,¹⁰ providing an example of the importance of the drug–polymer composition.

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The biodegradable poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA) polymers are widely used as the lipophilic parts in PEG-PLA and PEG-PLGA (PEG = poly(ethylene glycol)) nanoparticles and polymer micelles, respectively. These polyesters degrade into nontoxic lactic and glycolic acids, respectively, which can be metabolized to carbon dioxide and water.¹¹ PEG chains of certain molar masses, between 2000 and 30 000 g/mol,¹² are preferentially used for the hydrophilic part. Because of their low level of protein absorption, they facilitate long circulation times for the drug carriers in the bloodstream in intravenous applications,¹³ targeting the pathological site by the enhanced permeability and retention (EPR) effect,¹⁴ and finally being eliminated by renal and hepatic pathways.¹² Lipophilic anticancer drugs, such as sagopilone¹⁵ or paclitaxel,¹⁶ were incorporated into poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA) micelles with clinically relevant drug concentrations. Recently, it was shown that PEG-*b*-PLA micelles can deliver several poorly water-soluble drugs simultaneously.¹⁷ Paclitaxel, etoposide, docetaxel, and 17-(allylamo)-17-(demethoxy)geldanamycin were incorporated individually and in combinations. It was reported that the drugs' solubilities in micelles were higher than those in pure water. In addition, the presence of multiple drugs did not influence the incorporation of any of them.

In the past few years our group has developed a novel PEG-poly(hexyl-substituted PLA) (PEG-hexPLA) excipient, wherein the methyl groups of PLA are substituted by hexyl groups, which increases the lipophilicity of the micelle core and facilitates the incorporation of many potential lipophilic drugs.^{18,19} Indeed, Trimaille et al. showed that the rate of lipophilic drug incorporation into the more lipophilic PEG-hexPLA micelle core is significantly higher than in comparable PEG-PLA polymer micelles.

As mentioned above, the preparation of pharmaceutical formulations with sufficient lipophilic drug loadings is a major challenge. A recent doctoral thesis (by Karin Mondon) reported remarkable drug incorporation efficiencies, which were experimentally achieved in MPEG-hexPLA polymer micelles in comparison to MPEG-PLA micelles. This efficiency could not be adequately explained by considering simply the compounds' characteristic parameters, such as their molar masses, water solubilities, log *P* values, available proton donor/acceptor groups, and surface tensions, or their combinations. Thus, a deeper understanding of the incorporation phenomenon of lipophilic drugs into these polymer micelles is desirable.

Today, computer simulation methods are a powerful means of determining component properties on the basis of their chemical structures, and they can be used, for example, to assess the compatibility of drugs with excipients in formulations such as aqueous solutions, emulsions, granules, or microspheres.^{20–25} Huynh et al. used semiempirical methods and molecular dynamics (MD) simulations to calculate the solubility of some poorly water-soluble drugs in small-molecule liquids.²¹ These investigators demonstrated that MD simulations can be a reliable method for this type of calculation. Computer methods were also used to predict particle structures on the basis of drug-polymer interactions. Long et al. calculated such drug-polymer interactions by a dissipative particle dynamic simulation method for the prediction of a solid lipid particle structure.²⁶

In the present work, MD simulations were applied and evaluated as a support for the understanding of the formulation of poorly water-soluble drugs with novel polymer micelles. A novel simulation approach was adopted to characterize the

interactions between the amphiphilic copolymer excipient PEG-hexPLA and four different lipophilic drug candidates by Flory-Huggins interaction parameters. The Flory-Huggins parameters obtained from MD simulations were compared with the data from experiments. Theoretical and experimental results demonstrated an extremely consistent trend which validates the molecular dynamics simulation approach adopted for this study and opens the possibility to apply it to other polymer micelle systems of interest.

2. MATERIALS AND METHODS

2.1. Polymers and Drug Formulation. 2.1.1. Materials.

Griseofulvin, ketoconazole, and quercetin dehydrate were purchased from Sigma (Buchs, Switzerland), and tetrahydrofuran (THF) was purchased from SDS (Toulouse, France). Methoxypoly(ethylene glycol) (MPEG) with a molar mass of 2000 g/mol was supplied by Union Carbide Corp. (Houston, TX). Tin(II) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) and acetone p.a. were purchased from Aldrich (Buchs, Switzerland) and Fluka (Buchs, Switzerland), respectively, and were used as received. The monomer hexyl-substituted lactide (hexLA) and the derived polymers (hexPLA) were synthesized as described in a previous paper.¹⁸

2.1.2. Synthesis and Characterization of the MPEG-hexPLA Block Copolymers. The synthesis of the MPEG-hexPLA block copolymers with a molar mass of approximately 5000 g/mol has been described previously.^{3,18} Briefly, MPEG-hexPLA block copolymers were synthesized by ring-opening polymerization of hexLA with MPEG of 2000 g/mol molar mass as the initiator and $\text{Sn}(\text{Oct})_2$ as the catalyst. The obtained block copolymers were characterized for their molar mass (M_n) and polydispersity index (PI) by gel permeation chromatography (GPC). The GPC setup was composed of a Waters system with Waters Styragel HR1-3 columns and a Waters 410 differential refractometer (Waters, Milford, MA). The analyses were carried out using polystyrene (PS) of different molar masses as calibration standards (PSS, Mainz, Germany).

2.1.3. Preparation of the Drug-Loaded MPEG-hexPLA Micelle. Drug-loaded MPEG-hexPLA micelle solutions were prepared by the cosolvent evaporation method described by Mondon et al.²⁷ Briefly, 6 mg of drug and 20 mg of copolymer were dissolved in 2 mL of acetone or THF. The organic mixture was added dropwise with stirring to 4 mL of ultrapure water using a peristaltic pump. Subsequently, the organic solvent was slowly removed by evaporation at 200 mbar. Final micelle concentrations were adjusted to 5 mg_{copolymer}/mL by adding ultrapure water. After overnight equilibration, the solutions were centrifuged at 9500g for 15 min to remove nonincorporated undissolved drug.

2.1.4. Quantification of Drug Loading in MPEG-hexPLA Micelles. Drug loadings in MPEG-hexPLA micelles were determined after centrifugation to remove nonincorporated and precipitated non-water-soluble drug compound. The supernatant was diluted in acetonitrile at a ratio of 1:10 to break up the micelles and to release the drug for quantitative analysis. The drug loading quantification was then performed by HPLC.

The drug content (DC) and the drug solubility in the micelle (DM) were calculated using the following equations:

$$\begin{aligned} \text{drug content} & (\text{mg}_{\text{drug}}/\text{g}_{\text{copolymer}}) \\ &= \frac{\text{mass of drugs incorporated into micelles}(\text{mg})}{\text{mass of copolymer used}(\text{g})} \end{aligned}$$

$$[\text{drug}]_{\text{micelles}} (\text{mM}) = \frac{\text{DC}(\text{mg drug/g copolymer}) \times \text{concentration of copolymer(g/L)}}{\text{molar mass of the drug(g/mol)}}$$

2.2. Computer Simulations. 2.2.1. Flory–Huggins Interaction Parameters for Drug–Polymer Interactions.

The miscibility of two polymers is reliably described by the Flory–Huggins (FH) theory. Originally, this theory was used to describe polymer–polymer and polymer–solvent interactions.²⁸ This theory is based on the lattice theory and comprises the mixing of two components as a process determined by a combination of the enthalpy and entropy factors.²⁹ The mixing is described by the change in the Gibbs free energy:

$$\Delta G_{\text{mix}} = RT[n_1 \ln \varphi_1 + n_2 \ln \varphi_2 + n_1 \varphi_2 \chi_{\text{FF}}] \quad (1)$$

where R is the gas constant, T is the temperature (K), n_i and φ_i are respectively the number of moles and the volume fraction, and γ_{FH} is the Flory–Huggins interaction parameter.

The first two terms describe the entropy of mixing, which is always unfavorable. The χ_{FH} parameter is dimensionless and characterizes the strength of the interaction between the molecules of the solvent and of the solute. The two components will be miscible if the χ_{FH} parameter is negative or tends to zero. The critical value of χ_{FH} , above which two components are immiscible, is 0.5.

The groups of Lavasanifar³⁰ and Allen^{31,32} calculated χ_{FH} for drug-exipient pairs by using the semiempirical group contribution method (GCM); this method takes only the lipophilic part of the copolymer into consideration. However, the hydrophilic part could in general also interact with the drug molecules. Moreover, drug molecules could be located in different positions in the micelles depending on their hydrophilic/lipophilic character throughout the whole length of the copolymer chain: in the core, at the interphase of the lipophilic and hydrophilic blocks, or in/on the corona of the micelles.

Recently, it was shown that χ_{FH} can be calculated by MD simulations.^{21,33,34} The atomistic resolution allows for a more reliable representation of the interior of the micelle due to the possibility to calculate the interactions of a drug molecule with the whole polymer chains.

Huynh et al. successfully calculated Flory–Huggins interaction parameters using the energy of mixing ΔE_{mix} of a drug and copolymer.²¹ Previously, it was also shown that the change of the system volume with mixing is insignificant; therefore, ΔE_{mix} can be replaced with ΔH_{mix} .³³ For this reason, we chose to calculate χ_{FH} by the following equation:

$$\chi_{\text{FH}} = \frac{V_{\text{ref}} \Delta H_{\text{mix}}}{RT} \quad (2)$$

where V_{ref} is the smallest volume among the molar volumes of drugs and that of the repeating units of the block copolymer in the drug–polymer mixture and ΔH_{mix} is the enthalpy of mixing.

The enthalpy of mixing ΔH_{mix} was calculated from the enthalpy of binding ΔH_{bind} of the pure drug, pure copolymer, and drug–copolymer mixture, as outlined in the following equation:

$$\Delta H_{\text{mix}} = \varphi_1 \left(\frac{\Delta H_{\text{bind}}}{V} \right)_1 + \varphi_2 \left(\frac{\Delta H_{\text{bind}}}{V} \right)_2 - \left(\frac{\Delta H_{\text{bind}}}{V} \right)_{1-2} \quad (3)$$

where φ_1 and φ_2 are the volume fractions of components 1 and 2, V is the total volume of the system, and ΔH_{bind} is the enthalpy of binding.

The Flory–Huggins interaction parameter χ_{FH} can be calculated directly from the enthalpy of binding ΔH_{bind} obtained from MD simulation trajectories. The calculated χ_{FH} opens the possibility of assessing the drug “solubilization” within the polymer micelles, corresponding to the drug incorporation.

2.2.2. MD Simulation Approach. **2.2.2.1. Creation of the Molecular Models.** The models for the linear MPEG–hexPLA

block copolymer were created with the Material Studio 5.0 software.³⁵ The MPEG block polymer subunit (2000 g/mol) was composed of 44 ethylene glycol monomers, whereas hexPLA (3000 g/mol) consisted of 10 corresponding ring-opened hexLA monomers (Figure 1). All of the MD simulations

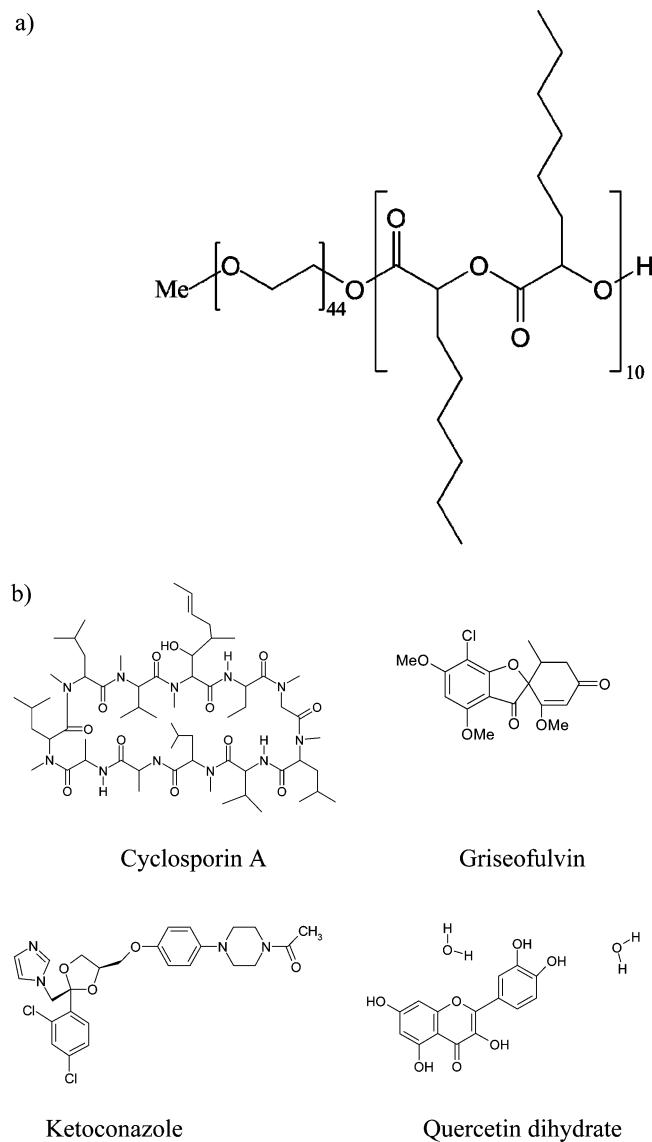


Figure 1. Chemical structures of (a) MPEG–hexPLA and (b) the drugs.

were run using the AMBER 10 software suite of programs.³⁶ The monomers that compose the linear block copolymer were parametrized using the “general AMBER force field (GAFF)” (gaff.dat).³⁷ The AM1-BCC^{38,39} calculation method within the antechamber [40] module of AMBER 10 was used to compute the partial charges according to a well-validated procedure that was adopted in previous studies by our group on other

Table 1. Chemical Structures and Solubilities of the Drugs in Pure Water and in MPEG–hexPLA Micelle Solutions and Molecular Dynamics Simulation Results

drug	log <i>P</i>	experimental data			MD simulation		
		drug solubility in water (mM)	drug solubility in micelle solution (mM)	drug content per polymer (mg/g)	ΔH_{mix} (kcal cm ⁻³)	χ_{FH}	drug content per polymer (mg/g)
cyclosporin A ($M_w = 1202.61$ g/mol)	8.20 ^a	0.010 ^a	1.100 ^e	321.0 ^e	-16.586	-1.080	318.2
griseofulvin ($M_w = 352.77$ g/mol)	3.18 ^b	0.060 ^a	0.809	166.0	-9.182	-0.598	176.4
ketoconazole ($M_w = 531.48$ g/mol)	4.35 ^c	0.032 ^d	0.923	55.4	-2.720	-0.177	52.6
quercetin dihydrate ($M_w = 338.28$ g/mol)	1.48 ^c	0.177 ^c	0.182	12.5	-0.434	-0.028	8.8

^aValues experimentally obtained by P.-A. Carrupt, A. Guillot, and S. Martel (Pharmacochimistry, University of Geneva, Geneva, Switzerland; personal communication). ^bOSPAR Commission, 2005, http://www.ospar.org/documents/dbase/publications/p00199_BD%20on%20clotrimazole.pdf.

^cChemIDplus Advanced, <http://chem.sis.nlm.nih.gov/chemidplus/>. ^dBioAqueous Solubilization Services, Dowpharma, <http://www.dow.com/webapps/lit/litorder.asp?filepath=pharma/pdfs/noreg/715-00011.pdf&pdf=true>. ^eMondon, K.; Zeisser-Labouèbe, M.; Gurny, R.; Möller, M. *Eur. J. Pharm. Biopharm.* **2011**, *77*, 56–65.

hyperbranched polymers (dendrons^{41–44} and dendrimers^{45–49}). The same procedure was adopted for the parametrization of the lipophilic drugs cyclosporin A, griseofulvin, ketoconazole, and quercetin dehydrate. The MPEG–hexPLA systems were composed of 16 linear chains of MPEG–hexPLA block copolymers and a number of drug molecules, which were chosen and calculated on the basis of experimental mean stoichiometries. The 16 polymer chains were disposed randomly and uniformly in space. The suitable numbers of drug molecules were inserted in the cavities between polymer chains to reproduce the experimental drug/polymer mass ratio (mg/g, reported in Table 1).

The drug–polymer mixtures (see, for instance, Figure 4, the cyclosporin A–MPEG–hexPLA case) were initially equilibrated in a vacuum by running a 10 ns MD run at 300 K to obtain a first preliminary compaction of the systems. Further, a truncated-octahedric periodic box was constructed around the compacted complexes (with a 0 Å buffer constant in the three dimensions) using the leap module of AMBER 10. The voids, mostly focused in correspondence to the truncated octahedron corners, were filled with TIP3P explicit water molecules.⁵⁰ The micellization process is often conceived as a very uniform process, where all of the lipophilic parts of the polymer chains compose the interior of the micelle, while the hydrophilic portions form the micelle surface. This however is a simplification. In fact, during the mixing process multiple nucleation spots would be present in a real solution due to the rapid tendency of the flexible polymer to cover the lipophilic zones with the PEG chains. These multiple agglomerates would aggregate further to form the whole micelle. In view of this, a micelle would appear as a tangled ball-shaped structure rather than an almost perfect starlike oriented assembly, as it is often represented. The micelle core would then be far from a uniform structure composed only by lipophilic molecules, but would be composed by a crowded compacted ravel of polymer chains and drug molecules. Also a small amount of water molecules entrapped during the compaction process can occasionally be present in the core. The molecular model we have created and used in this study was aimed to account for this situation in the micelle core.

2.2.2.2. Production-Phase MD Simulations. The four drug–MPEG–hexPLA block copolymer systems, composed of polymer chains, drug molecules, and explicit water molecules, were first minimized and then heated for 50 ps in isothermal–isochoric (NVT) conditions and equilibrated further for another 50 ps in isothermal–isobaric (NPT) conditions, respectively, to reach the relevant experimental temperature of 300 K and to relax the density of the systems. All of the drug–copolymer

systems were then equilibrated for 10 ns under NPT periodic boundary conditions at 300 K and 1 atm using a time step of 2 fs, the Langevin thermostat, and an 8 Å cutoff. The particle mesh Ewald (PME) approach⁵¹ was adopted to treat long-range electrostatic effects, and the SHAKE algorithm was used to constrain all of the bonds that involve hydrogen atoms.^{52,53}

Each of the MD runs was executed using the sander and the pmemd modules within the AMBER 10 software suite and the parm99 all-atom force field by Cornell et al.⁵⁴ Simulations were conducted working in parallel (up to 128 CPUs) on the Cray XT5 at the CSCS Swiss National Supercomputer Center of Manno (Switzerland) and on the 64 cores of the 16 Intel Core2 Quad Q9450 2.66 GHz 1333 MHz calculation cluster of the University of Geneva.

2.2.2.3. Energetic and Structural Analyses. The calculation of the enthalpy of binding ΔH_{bind} was based on the MM-GBSA approach.⁵⁵ For each system, 200 unbound drug–copolymer complex snapshots were taken from the equilibrated phase of the MD trajectories. ΔH_{bind} was calculated as follows:

$$\Delta H_{\text{bind}} = \Delta E_{\text{int}} + \Delta G_{\text{sol}} \quad (4)$$

The enthalpic contribution was calculated by summing the in vacuo gas-phase energies (eq 5) and the solvation free energies (eq 6):⁵⁶

$$\Delta E_{\text{gas}} = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} \quad (5)$$

$$\Delta G_{\text{solv}} = \Delta G_{\text{GB}} + \Delta G_{\text{NP}} \quad (6)$$

The generalized-Born (GB) approach⁵⁷ was used to calculate the polar component ΔG_{GB} , while the nonpolar contribution ΔG_{NP} of the solvation energy ΔG_{solv} was calculated as follows:

$$\Delta G_{\text{NP}} = \gamma(\text{SASA}) + \beta \quad (7)$$

where $\gamma = 0.00542$ kcal/Å² and $\beta = 0.92$ kcal/mol. The solvent-accessible surface area (SASA) was estimated with the MSMS program.⁵⁸ The resulting ΔH_{mix} values were further compared to solubility data obtained from experiments.

3. RESULTS AND DISCUSSION

As outlined in the Introduction and in the computational methods section (section 2.2), semiempirical methods, based on the sum of the involved molecules' structural group contributions, have already been used for calculating solubilities of some poorly water-soluble drugs in liquids. In other cases, drug interactions with polymers or parts of polymers were simulated by molecular dynamics simulations, in which, however, neither the hydrophilic polymer parts nor the presence of water

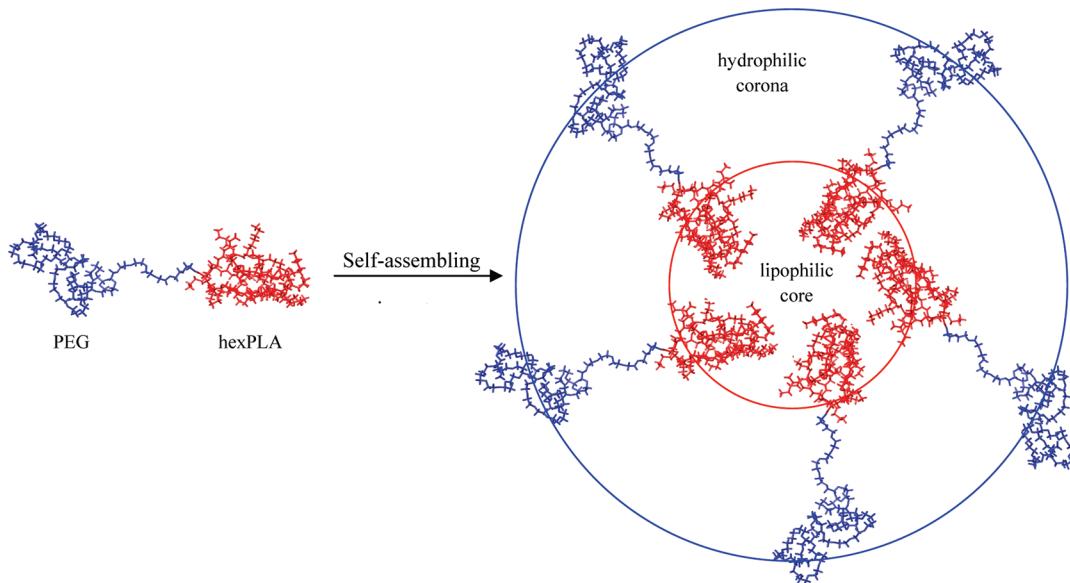


Figure 2. Conceptual scheme of MPEG–hexPLA self-assembling into a micelle structure in an aqueous environment. Hydrophilic MPEG is colored in blue and lipophilic hexPLA in red.

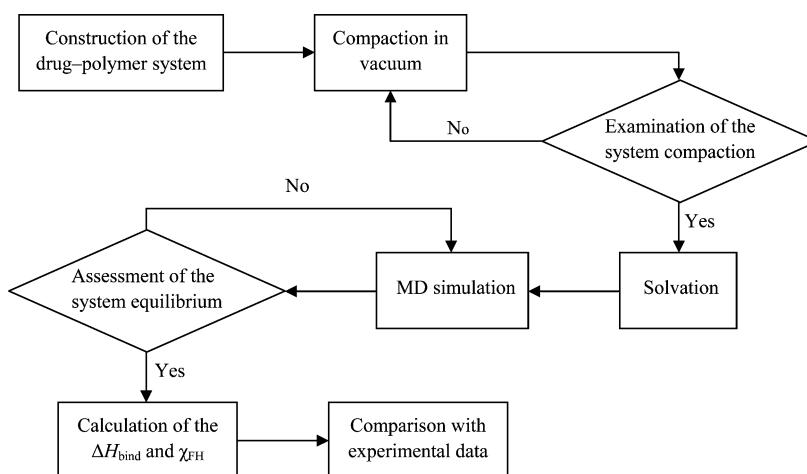


Figure 3. Strategy of MD simulation for the assessment of drug incorporation into micelles.

molecules was considered. In this work, MD simulation was used to characterize micelle formation and the interactions between polymers and drugs in more detail. This study was aimed to provide a more exhaustive snapshot of what happens in the interior of the micelle. Atomistic simulation provides an estimation of the incorporation abilities of drugs into the micelle in aqueous solution by studying the thermodynamic stability of the drugs inside the polymer matrix in a time-dependent manner. For this purpose, it is important that the whole amphiphilic diblock copolymer chains, as well as a small amount of water molecules, as discussed in section 2.2.2, are taken into account.

The first aim of this study was the validation of this new MD simulation procedure, which was tested on four drug–polymer systems: (a) cyclosporin A–MPEG–hexPLA, (b) griseofulvin–MPEG–hexPLA, (c) ketoconazole–MPEG–hexPLA, and (d) quercetin dehydrate–MPEG–hexPLA (for the initial structures of MPEG–hexPLA and the drugs and MPEG–hexPLA's compact micellization, see Figures 1 and 2, respectively). The drugs were chosen for their significantly different water solubilities and $\log P$ values (Table 1), ranging from the lowest water

solubility, 0.010 mM for cyclosporin A, to the highest, 0.177 mM for quercetin dehydrate.

Prior to the MD simulations, the experimental incorporation data were evaluated. Griseofulvin, ketoconazole, and quercetin dehydrate were incorporated into the MPEG–hexPLA micelles by a cosolvent evaporation method. The data for cyclosporin A were taken from the literature.²⁷ The obtained solubility values for these drugs in MPEG–hexPLA micelles are presented in Table 1. It can be seen that the solubilities of cyclosporin A, griseofulvin, and ketoconazole increase significantly by formulation in the polymer micelles, whereas the solubility of quercetin dehydrate remains nearly the same. This difference can be contributed to the lipophilicity of these drugs. Because cyclosporin A ($\log P = 8.2$), griseofulvin ($\log P = 3.18$), and ketoconazole ($\log P = 4.35$) are considerably more lipophilic than quercetin dehydrate ($\log P = 1.48$), they should interact better with the lipophilic polymer in the micelle core, thus resulting in higher incorporation rates. However, in the case where two drugs have a similar $\log P$ value (griseofulvin and ketoconazole), significantly different solubilities were obtained, whereby griseofulvin with the lower $\log P$ has, surprisingly, the

higher solubility in the micelles, indicating one more time that $\log P$ cannot be taken as the only solubilization parameter.

In our computational work, the Flory–Huggins interaction parameters χ_{FH} of the four drugs with reference to the polymer micelle were calculated from the MD trajectories. This concept is analogous to the χ_{FH} calculations of solutes in classical solvents, whereby χ_{FH} values which tend to zero, or even have consistent negative values, identify drug solubilization (potential drug incorporation into the polymer micelles); in general, the more negative the χ_{FH} value, the better the solubilization and the higher the incorporation into the micelles.

For the MD simulations, we constructed the specific drug–polymer systems which underwent a preliminary compaction step. After this step, a truncated octahedral periodic box was constructed around the compacted drug–polymer complexes (with a 0 Å buffer) and the residual voids were filled with water molecules; a certain amount of solvent molecules are supposed to be present, also in reality, due to entrapment occurring during micellization. These molecular systems are aimed to reproduce the complexation and the compaction present in the interior part of the MPEG–hexPLA diblock copolymer micelles with different drugs (Figures 4 and 5). MD simulations were

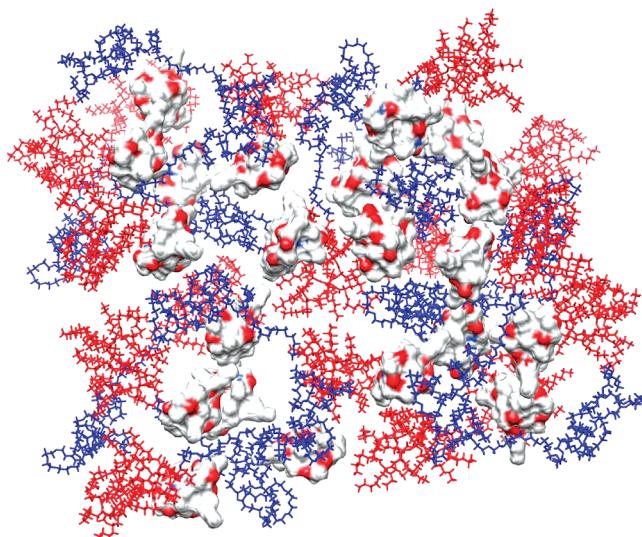


Figure 4. Snapshot of the initial “open configuration” of the cyclosporin A–MPEG–hexPLA system. The 21 cyclosporin A molecules are presented as van der Waals surfaces. Within the MPEG–hexPLA block copolymer, the PEG polymer part is colored in blue and the hexPLA part is in red.

then run to calculate the specific Flory–Huggins interaction parameters χ_{FH} from the enthalpies of complexation as expressed in eq 2. This approach allows for direct estimation of the drug solubility in the polymer environment based on the thermodynamic estimation of energetic interactions between drug molecules and the matrix.

Because the simulation time depends heavily on the number of atoms in the system, a base system was created that comprised 16 equally spaced linear chains of the MPEG–hexPLA diblock copolymer. Importantly, the number of polymer chains was maintained as a constant for all four different drug–polymer systems. To reproduce the correct drug–polymer stoichiometry inside the micelles, the suitable number of drug molecules was chosen in accordance with the correct drug content present in the micelle formulations determined experimentally (Table 2,

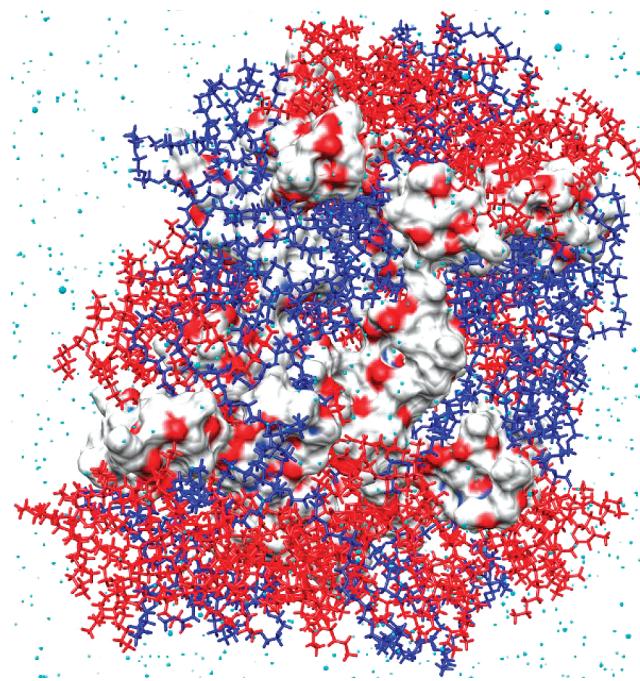


Figure 5. Snapshot taken from the production-phase MD run of the cyclosporin A–MPEG–hexPLA periodic system. The 21 cyclosporin A molecules are represented as van der Waals surfaces. Within the MPEG–hexPLA block copolymer, the PEG polymer part is colored in blue and hexPLA in red. Water molecules are represented in cyan (the hydrogen atoms of water are omitted for clarity).

Table 2. Stoichiometry of the Complexation of Different Drugs with MPEG–hexPLA^a

complex	no. of MPEG–hexPLA chains	no. of drug molecules ^b	molar ratio present in micelles ^c
cyclosporin A	16	21	0.76
griseofulvin	16	4	0.86
ketoconazole	16	15	0.95
quercetin dihydrate	16	2	0.99

^aThe stoichiometries of the bulk systems are calculated to reproduce the average drug/polymer ratio that is present in the micelles. ^bThe number of drug molecules varies depending on the experimental drug content. ^cThe molar ratio is defined as the ratio between the number of polymer chains and the number of drug molecules in the bulk model of the micelle.

drug/polymer mass fraction, mg/g). Drug molecules were added randomly and uniformly in the empty cavities between the polymer chains to guarantee an almost equal spacing (Figure 4). To reproduce the compaction that is present in the bulk of the micelles, drug–MPEG–hexPLA systems were initially equilibrated in vacuum for 10 ns of MD simulation, which led to sufficient compactations. This preliminary gas-phase step was done to decrease the computational time needed to achieve a satisfying compaction (starting from an “open” fully solvated situation would have required an unreasonably high simulation time) and to decrease the number of atoms in the system in the second step of our procedure. The obtained compacted systems were considered as the solute part of a portion of the interior of a micelle. Afterward, TIP3P explicit water molecules were added, allowing for the creation of a periodic box containing the solute drug–polymer systems together with a small amount of water molecules present at the corners. As said, this amount of water is assumed to be present in a small quantity also in the bulk of the

real micelles due to the entrapment that occurs during the compaction phase. Moreover, this small amount of solvent was useful to guarantee the stable density that is assumed to be present at the core of the micelle. By this the four molecular periodic systems of cyclosporin A–MPEG–hexPLA, griseofulvin–MPEG–hexPLA, ketoconazole–MPEG–hexPLA, and quercetin dehydrate–MPEG–hexPLA were obtained (in Figure 5 cyclosporin A is reported as an example).

The production-phase MD simulation lasted for 10 ns in NPT conditions at 300 K. During this time all the systems reached equilibrium with good stability. The last 2 ns of each MD run was used for the energetic analysis. The enthalpy of mixing ΔH_{mix} and then the Flory–Huggins interaction parameter χ_{FH} were obtained by means of eqs 2 and 3 for every drug–polymer combination. The calculated χ_{FH} values for cyclosporin A–MPEG–hexPLA, griseofulvin–MPEG–hexPLA, ketoconazole–MPEG–hexPLA, and quercetin dehydrate–MPEG–hexPLA were -1.080 , -0.598 , -0.177 , and -0.028 , respectively. As described above, a drug is considered to be solubilizable in the polymer micelles for values of $\chi_{\text{FH}} \leq 0$. Thus, the data from the modeling show that MPEG–hexPLA polymer micelles should have the ability to incorporate the four different drugs. This is fully confirmed by the experiments. Importantly, also the trend in the calculated absolute χ_{FH} values reflects the experimental reality; i.e., cyclosporin A shows the lowest χ_{FH} value which corresponds to the highest incorporated drug amount into the micelle, and vice versa, drugs with less negative χ_{FH} values have the lowest drug incorporation (Table 1).

Figure 6 presents a plot of the calculated χ_{FH} parameters with respect to the experimental micellar drug loading

These results demonstrate the exceptional reliability of this method for the revision of drug solubility into a determined polymer micelle and allow us to assume that the presented strategy could be transferred onto other polymer micelle–drug systems.

4. CONCLUSIONS

A computer simulation method able to assess the ability of polymer micelles to incorporate poorly water-soluble drugs provides considerable benefits with respect to laborious, time-consuming, and expensive experiments. Here, we have reported on a novel and reliable molecular dynamics simulation approach to estimate the solubility of lipophilic drugs into polymer micelles. This procedure is based on the creation and simulation of molecular systems aimed to reproduce a portion of the interior of the real micelle, where polymers, drugs, and a small amount of water molecules are tightly wrapped and compacted together. Simulations allow for the calculation of the Flory–Huggins interaction parameter χ_{FH} , which estimates the solubilization of the drug into the polymer micelle from the enthalpy of mixing ΔH_{mix} . This provides immediate insight into the drug incorporation ability into the micelles, which finds exceptional consistency with the experiments (measured micelle drug/polymer mass fraction, mg/g). The calculated χ_{FH} values were validated with experimental data for four different drugs with significantly different lipophilicities. The relationship between χ_{FH} from MD simulation and the drug/polymer real mass fraction determined with experiments showed an almost perfect linear trend, demonstrating the level of reliability of this approach.

This presented procedure could be used to estimate the solubility of a variety of lipophilic drugs into polymer micelles once the fitting with a few test cases has been verified and proven. In addition, due to the versatility of MD simulation and of the procedure adopted herein, it allows for direct estimation of the effect of structural modifications of the polymer chains which form the micelles and the efficiency of drug incorporation. Future efforts will follow these directions.

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Notes

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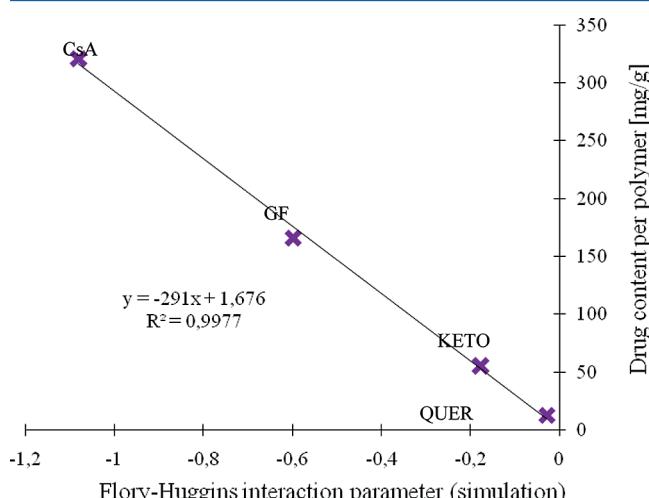


Figure 6. Relationship between the Flory–Huggins interaction parameter χ_{FH} calculated from MD trajectories, and the micellar drug loading, in terms of the drug/polymer mass ratio (mg/g) determined from experiment. Key: CsA, cyclosporin A; GF, griseofulvin; KETO, ketoconazole; QUER, quercetin dehydrate; \times , experimental drug content per polymer (mg/g).

(drug/polymer mass fraction, mg/g). This graph demonstrates an almost perfect linear trend ($R = 0.9977$) between theory and experiments for the tested systems. The relationship between the χ_{FH} parameters and experimental drug contents per polymer is expressed by

$$\text{drug content(mg}_{\text{drug}}/\text{g}_{\text{copolymer}}\text{)} = -291\chi_{\text{FH}} + 1.676$$

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