Doxorubicin and β-Lapachone Release and Interaction with Micellar Core Materials: Experiment and Modeling

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Polymer micelles with two different core-forming blocks, poly(D,L-lactide) (PLA) and poly(ε-caprolactone) (PCL), but the same coronal material, poly(ethylene glycol) (PEG), were investigated in this study as nanoscopic drug carriers. The release of two different drugs, doxorubicin (DOX) and β-lapachone (β-lap), from PEG(5k)-b-PCL(5k) and PEG(5k)-b-PLA(5k) micelles was studied at pH 5.0 and 7.4. Mathematical solutions of both Higuchi's model and Fickian diffusion equations were utilized to elucidate the differences between the micelle core materials for the two drugs. The neutral and smaller of the two drugs tested, β-lap, demonstrated faster, pH-independent release, suggesting that no substantial changes occurred in either micelle core at lower pH. In contrast, the release rate of DOX was found to noticeably increase at lower pH with a larger cumulative amount of drug released. Different core materials were shown to have considerable influence on the release kinetics of both drugs: in both cases, the more hydrophobic PCL core showed slower drug release rates compared with the less hydrophobic PLA core. Exp Biol Med 232:1090–1099, 2007

Key words: polymer micelles; drug release; drug-polymer interactions; mathematical modeling; physicochemical properties

Introduction

In recent years, polymer micelles have received significant attention as a promising nanomedicine platform for pharmaceutical delivery. Polymer micelles have shown the ability to efficiently solubilize hydrophobic agents and improve drug pharmacokinetics (1–7) and have in several cases reached clinical trials (8, 9). These small particles (<150 nm diameter) not only increase drug solubility, but also passively target tumor tissues by preferentially accumulating into the "leaky" tumor vasculature *via* the enhanced permeation and retention effect (10–12). The well-defined chemistries used to create these structures also allows their customization with additional functionalities, such as temperature sensitivity and ligand targeting (7, 13).

Despite their considerable therapeutic promise, many aspects of micellar drug delivery remain to be fully characterized and understood. Among them, the release behavior of the drug from micelles is of extreme importance for drug bioavailability and efficacy. There are numerous reports of different drug release studies from various micellar systems. However, direct comparison of the results from these studies is often cumbersome, as conditions of micelle fabrication, drug encapsulation, and, most importantly, drug release conditions vary. Among the recent studies that investigate the influence of different factors on drug release are the reports by Kataoka *et al*. on the pH dependence of DOX release from poly(ethylene glycol)-*b*-poly(β-benzyl-L-aspartate) block copolymer (PEG-*b*-PBLA) micelles (14), the analysis of the influence of core additives on drug encapsulation and release of rapamycin by PEG-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) micelles by Forrest *et al*. (15), and the comprehensive study of drug-polymer interactions for ellipticine and PEG-*b*-PCL or PEG-*b*-poly(D,L-lactide) (PEG-*b*-PLA) micelles performed by Liu *et al*. (16). One of the important conclusions made in these reports is that interactions between the drug and core-forming material or between drug and release media is of

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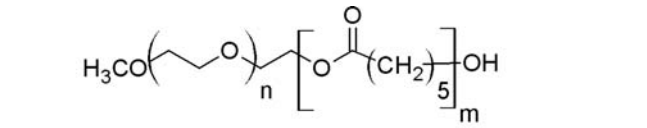
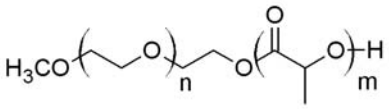
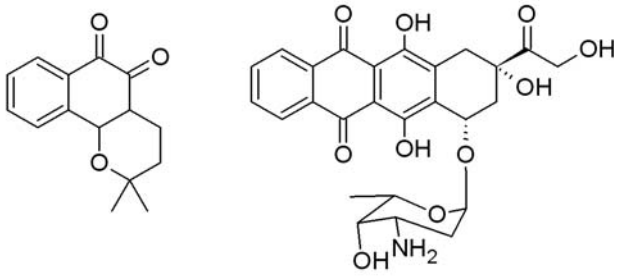


Poly(ethylene glycol)-*b*-poly(ε -caprolactone)
PEG-*b*-PCL



Poly(ethylene glycol)-*b*-poly(D,L-lactide)
PEG-*b*-PLA



β-Lapachone (β-Lap) Doxorubicin (DOX)

Figure 1. Chemical structures of PEG-*b*-PCL, PEG-*b*-PLA, β-lap, and DOX.

great importance to drug release by polymeric micellar systems. Of course, there might be an influence of the release media on the polymeric material itself, so these two effects may be interconnected. In this report, we attempt to analyze these effects by studying release of two different anticancer drugs, doxorubicin (DOX) and β-lapachone (β-lap) from two polymer micelles, PEG-*b*-PCL and PEG-*b*-PLA (Fig. 1). Mathematical modeling of the release behavior is employed to quantify the importance of such factors as core hydrophobicity and drug solubility on drug release. This complex experimental-analytical approach will help to identify important factors that influence drug release.

DOX (Fig. 1) was chosen as one of the most common chemotherapeutic drugs used in micellar formulations (8, 17–19), with DOX-encapsulated micelles being one of the few systems that reached clinical trials (8). DOX is known to have a pH-dependent solubility (20), unlike the neutral β-lap (Fig. 1) (21). By comparing the pH dependences of release for these two compounds, we will gain insight into any pH dependence of the micelle release properties themselves. Both micelles, PEG-*b*-PCL and PEG-*b*-PLA, are comprised of nontoxic and biodegradable polyesters that have been explored previously as micelle cores (13, 16, 17, 22–25). PCL is a semicrystalline polymer with a melting temperature (T_m) of around 55°C (26), whereas PLA is fully amorphous, with a glass transition temperature (T_g) of around 50°C (27). The semicrystalline and hydrophobic nature of PCL has been suggested to cause slow drug release (17), and we further hypothesize that an amorphous, less hydrophobic PLA core will result in faster release kinetics.

Drug release from a polymer matrix involves a multitude of processes, including possible matrix swelling, erosion or degradation of the polymers, drug dissolution, as well as external or internal mass transport of the dissolved drugs. To describe it exactly is a mathematically challenging task. A large number of analytical models have been put forward that use different assumptions to describe a particular process (28–30). These models are normally case specific, but could be combined to simultaneously address several factors (such as the polydispersity and crystallinity of polymers, particle size distribution [31, 32], and distribution of drugs in the matrix [31], as well as the entrapment of drugs in the matrix [32, 33], etc.). While such approaches could provide a complete picture of drug release, they require knowledge of multiple parameters of the system, which are often not readily available from experiments. As a result, the most commonly used models are those based on relatively simple mathematical approaches and that have the advantage of employing a small number of parameters. Among them, Higuchi's model (34) and Fickian diffusion–based approaches (35, 36) are the most commonly used. In our current study, we attempt to ascertain the effects of drug hydrophobicity, pH sensitivity, and the influence of the polymeric core on drug release. To serve this purpose, we employ both the Fickian diffusion–based approach and Higuchi's model, which allow us to take into account the difference in drug solubility and provide a quantitative measure of the kinetics of drug release. The comparison of the release behavior of different drug-polymer systems using equivalent modeling approaches will assist our fundamental understanding of their differences.

Materials and Methods

Materials. D,L-lactide was purchased from Alfa Aesar (Ward Hill, MA) and was purified by recrystallization from dried ethyl acetate and thoroughly vacuum dried for 24 hrs before use. Stannous (II) octoate ($Sn(Oct)_2$; Aldrich, St. Louis, MO) was used as received. ε-Caprolactone (ε-CL; Aldrich) and purified by vacuum distillation over calcium hydride. DOX in aqueous solution (DOX-HCl, 2 mg/ml) was purchased from the Bedford Laboratories (Bedford, OH), and was deprotonated at pH 9.6 to obtain the hydrophobic DOX. β-Lap was synthesized by Dr. William G. Bornmann from M. D. Anderson Cancer Center (Houston, TX). All organic solvents were of analytical grade. Toluene (Aldrich) was dried by refluxing over sodium and distilled under dry argon.

Synthesis of Methoxy-Terminated PEG-*b*-PLA

Copolymer. PEG-*b*-PLA was synthesized by ring-opening polymerization of D,L-lactide under dry argon at 110°C. Monomethyl ether hydroxyl (HO-PEG-OCH_3; number average molecular weight $M_n = 5000$ Da) was used as a macroinitiator. D,L-lactide was added as a monomer and stannous (II) octoate ($Sn(Oct)_2$) was added as a catalyst. After reacting for 4 hrs at 110°C, the mixture was allowed to cool to room temperature. PEG-*b*-PLA was purified by redissolving in tetrahydrofuran (THF) and precipitating in hexane three times. The overall yield was 95%. The degree of polymerization of the PLA was calculated by comparing the integral intensity of the characteristic resonance of the PLA at 5.2 ppm (–C(=O)-CH(–CH_3–)) and PEG resonance at 3.64 ppm (–OCH_2CH_2–) in the ^{1}H nuclear magnetic resonance (NMR) spectrum in chloroform ($CDCl_3$). The molecular weight and polydispersity index (PDI) of PEG-*b*-PLA were also characterized by gel permeation chromatography (THF as eluent), and the results were found to be consistent with ^{1}H NMR data. PEG-*b*-PLA ($M_n = 10.0$ kD; PDI = 1.2) was used in this study.

Synthesis of PEG-*b*-PCL Copolymer. The PEG-*b*-PCL copolymer was synthesized (with yields >95%) by ring-opening polymerization of ε-caprolactone under dry argon at 115°C for 24 hrs using PEG as a macroinitiator and $Sn(Oct)_2$ as a catalyst. The product was purified by precipitating twice into cold methanol from CH_2Cl_2 solution, and was then vacuum dried at 40°C. The block copolymer was characterized by ^{1}H NMR in $CDCl_3$ at room temperature. The degree of polymerization of the PCL block was calculated by comparing the integrals of the ^{1}H NMR characteristic peaks of the PCL block at 2.31 ppm (triplet, –C(=O)-CH_2–) and PEG block at 3.39 ppm (singlet, –OCH_2CH_2–). The molecular weight and polydispersity of PEG-*b*-PCL were also characterized by gel permeation chromatography (THF as eluent), and the results were found to be consistent with ^{1}H NMR data. PEG-*b*-PCL ($M_n = 10.0$ kD; PDI = 1.3) was used in this study.

Preparation of Drug-Loaded Micelles. Drug-containing polymer micelles were prepared as follows: 18 mg of PEG-*b*-PLA or PEG-*b*-PCL copolymer and 2 mg of drug (hydrophobic DOX was predissolved in 0.12 ml dimethylsulfoxide [DMSO]) were added to 1.08 ml THF in a glass vial. Next, the mixture was slowly added to 13 ml of water under sonication (60 Sonic Dismembrator; Fisher Scientific; Pittsburgh, PA). The mixture was vigorously stirred overnight to remove THF followed by filtration through a syringe filter (pore size 0.45 μm; Millipore, Billerica, MA) to remove large drug aggregates. Micelles were characterized by dynamic light scattering (see below). ^{1}H NMR was used to confirm the formation of core-shell structure. The strong resonance of methylene proton in PEG was detected, whereas all of the D,L-lactide or caprolactone proton resonances were hardly observed, demonstrating the core-shell structure of these micelles.

Drug-Loading Content Determination. The drug-loading content, defined as the weight percentage of DOX or β-lap based on the total micellar weight (i.e., weight of copolymer and drug) was quantified by UV-Vis analysis using a Lambda 20 spectrophotometer (Perkin-Elmer, Boston, MA). First, micelle solutions were frozen and lyophilized to yield the solid micelle samples. Then, the dried samples were weighed and redissolved in $CDCl_3$ for the β-lap micelles or a mixture of $CDCl_3$ and DMSO (1:1, v/v) for the DOX micelles followed by ultraviolet-visible spectroscopy (UV-Vis) analysis. The amount of loaded drug was determined based on the absorbance at 480 nm for DOX and at 257 nm for β-lap.

Dynamic Light Scattering (DLS). DLS was performed on a DLS Model 802 (Viscotek, Houston, TX). Scattered light was detected at an angle of 90° at room temperature and analyzed on an autocorrelator. Sample concentration during measurement was 1.4 mg/ml. The data for each sample was obtained in five independent measurements. The average hydrodynamic diameters and their standard deviations are provided in Table 1.

***In Vitro* Release of Drugs from Polymer Micelles.** The drug-loaded micelles were purified using Millipore centrifugal filters with a molecular weight cutoff of 100 kD to remove the free drug and to concentrate the samples in preparation for release studies. Approximately 15 mg of DOX-loaded polymeric micelles were placed into a total of 2 ml of water inside dialysis tubing, resulting in a micelle concentration of 7.5 mg/ml. The tubing was placed into 13 ml phosphate-buffered saline (PBS; pH 7.4) or acetate-buffered saline (pH 5.0) solutions. For β-lap release, 10 mg of drug-loaded micelles in water (2 ml), resulting in a micelle concentration of 5 mg/ml, were transferred into dialysis tubing (MW cutoff, 100 kDa). The tubing was placed into 8 ml PBS (pH 7.4) or acetate-buffered saline (pH 5.0) solutions. Release studies were performed at 37°C in a C24 Incubator Shaker (New Brunswick Scientific, Edison, NJ). At selected time intervals, all of the buffered solution outside the dialysis bag was removed for UV-Vis analysis and replaced with fresh buffer solution. The DOX and β-lap concentrations were calculated based on the absorbance intensity at 480 and 257 nm, respectively. Free drug transport from the dialysis tubing was studied under the same conditions as drug release from micelles, except the amount of the free drug was different: 1 ml solution of 2 mg/ml DOX-HCl at pH 5.0 and 2 ml of saturated deionized water solution (with 0.04 mg/ml of β-lap) were placed inside the dialysis tubing, respectively.

Mathematical Modeling. To simulate drug release kinetics from PEG-*b*-PCL and PEG-*b*-PLA micelles, we applied Higuchi's model (34). The advantage of this model over the Fickian diffusion model (35, 36) is that it accounts for the difference in solubility of the drugs in the buffer solution. As discussed below, this feature is especially important in understanding DOX release at different pH values.

The cumulative amount of drug released (SdQ) per unit time (dt) is given by the following equation:

Table 1. Summary of the Main Characteristics of Micelles Composed of PEG_{5000}-*b*-PCL_{5000} or PEG_{5000}-*b*-PLA_{5000} With or Without DOX or β-Lap Loading[a]

Drug	Micelle	Size without drug (nm)[b]	Drug-loading content (wt %)	Size with drug (nm)[b,c]
DOX	PEG-*b*-PCL	23.2 ± 2.2	3.80 ± 0.3	20.0 ± 3.3
	PEG-*b*-PLA	17.4 ± 1.2	2.32 ± 0.4	23.3 ± 4.5
β-lap	PEG-*b*-PCL	23.2 ± 2.2	1.0 ± 0.1	21.9 ± 2.4
	PEG-*b*-PLA	17.4 ± 1.2	0.7 ± 0.1	19.1 ± 2.1

[a] DOX, doxorubicin; PEG, poly(ethylene glycol); PCL, poly(ε-caprolactone); PLA, poly(D,L-lactide); β-lap, β-lapachone.
[b] Hydrodynamic diameter from dynamic light scattering.
[c] Average size (mean ± SD) = 21.075 ± 1.61 nm.

$$\frac{SdQ}{dt} = -4\pi a^2 \mathrm{D}\frac{dc}{da}, \tag{1}$$

where S is the surface area of the sphere exposed to the release medium, D is the diffusion constant of the drugs in the polymer matrix, and c is the concentration of drugs at radial distance, a, from the center of the sphere.

By assuming a pseudo–steady state at a moving front of the permeating fluids, the following equation is derived (34):

$$c_o(a_o^3 + 2a'^3 - 3a_o a'^2) + c_s\left(4a'^2 a_o + a_o^3 \ln\frac{a_o}{a'} - a_o^3 - a_o^2 a' - 2a'^3\right) = 6\mathrm{D}c_s a_o t, \tag{2}$$

where a_o is the radius of the spherical core of a micelle, a' is the distance of the moving front from the center of the core at time t, c_o is the drug-loading concentration, and c_s is the solubility of drug in the permeating fluids. The fractional drug released, $M(t)/M(\infty)$, is given by:

$$\frac{M(t)}{M(\infty)} = 1 - \left[\left(\frac{a'}{a_o}\right)^3 + \frac{1}{2}\frac{c_s}{c_o}\left(\left(\frac{a'}{a_o}\right) + \left(\frac{a'}{a_o}\right)^2 - 2\left(\frac{a'}{a_o}\right)^3\right)\right], \tag{3}$$

where $M(t)$ is the mass of drug released at time t and $M(\infty)$ is the amount of drug released as time approaches infinity.

Eqs. 2 and 3 are utilized to fit the experimental data. We note that in the case when the fit of the whole range of experimental data to the mathematical model is not possible (as for DOX release), the matching is optimized based on the initial period of the release only.

The effect of solubility of drugs on their release is reflected in Eqs. 2 and 3 by the ratio c_s/c_o. The drug-loading concentration, c_o, was estimated from the weight fraction of loaded drug and assuming the density of polymer in the core of the micelles to be 1 g/cm^3. The obtained values are listed in Table 2. The solubility of DOX at pH 7.4 was reported to be 0.0625 mg/ml (20). At lower pH, the degree of DOX protonation increases as does its solubility. The reported values range from 0.37 mg/ml at pH 5.0 in phosphate buffer (20) to 10–30 mg/ml for DOX-HCl (37, 38). The solubility of β-lap was measured to be 0.038 mg/ml and does not change with pH (21).

Knowing c_s/c_o , the ratio D/a_o^2 can be determined from fitting the experimental data. In order to obtain the diffusion coefficient, it is necessary to estimate a_o, the size of the micelle core. For a known hydrodynamic diameter (d) of the micelle (obtained by DLS), the core size was estimated as (Fig. 2):

$$a_o = d/2 - R_{corona}. \tag{4}$$

The thickness of the micelle corona (R_{corona}) could be estimated from the radius of gyration R_g of PEG (39):

$$R_{corona} \propto 2R_g = 2 \times 0.215 M_w^{0.583\pm0.031}\text{Å}, \tag{5}$$

where M_w is the average molecular weight of the PEG. The

Table 2. Release Characteristics of DOX and β-Lap from Micelles Composed of Diblock Copolymers of PEG_{5000}-*b*-PCL_{5000} or PEG_{5000}-*b*-PLA_{5000} at Different pHs[a]

Drug	Micelle	c_o (mg/ml)		c_s/c_o	D/a_o^2 (1/sec)	D (cm^2/sec)[b]
DOX	PEG-*b*-PCL	79.0	pH 5.0	9.44×10^{-3}	5.91×10^{-6}	1.13×10^{-18}
			pH 7.4	7.90×10^{-4}		
	PEG-*b*-PLA	47.5	pH 5.0	1.57×10^{-2}	9.54×10^{-6}	1.82×10^{-18}
			pH 7.4	1.32×10^{-3}		
β-lap	PEG-*b*-PCL	20.2		1.88×10^{-3}	7.78×10^{-5}	1.49×10^{-17}
	PEG-*b*-PLA	14.1		2.70×10^{-3}	1.27×10^{-4}	2.42×10^{-17}

[a] c_s, solubility of the drug in the bulk liquid phase, as required by Higuchi's model; c_o, drug-loading concentration in the micelle; D, diffusion coefficient of the drug in the core matrix; DOX, doxorubicin; PEG, poly(ethylene glycol); PCL, poly(ε-caprolactone); PLA, poly(D,L-lactide); β-lap, β-lapachone.
[b] Using average micellar core size $a_o = 4.37 \pm 0.33$ nm (mean ± SD) calculated using Eq. 4.

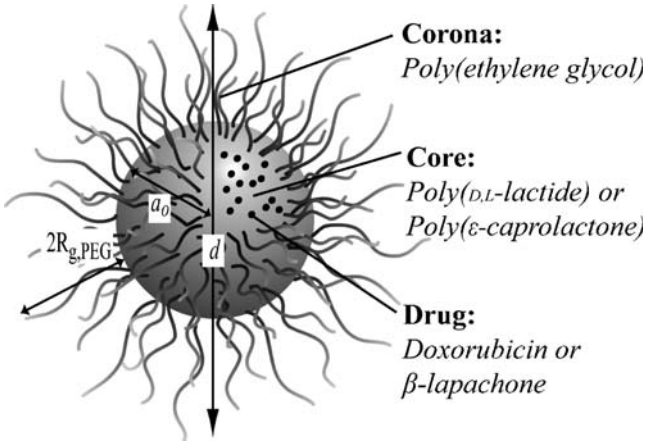


Figure 2. Schematic illustration of DOX or β-lap loaded diblock copolymer micelle with the same corona block PEG and two different core blocks poly (D,L-lactide) or poly(ε-caprolactone). *d,* hydrodynamic diameter of the micelle; $2R_{g,\ PEG}$, thickness of corona.

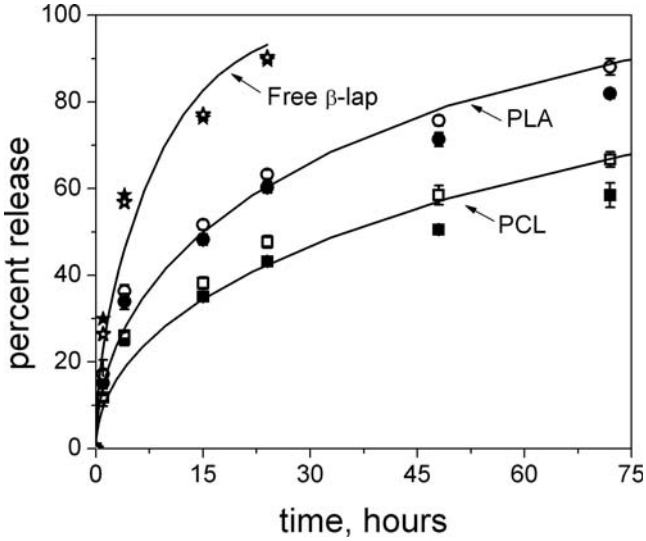


Figure 3. Cumulative release of β-lap versus time from two different micelles composed of diblock copolymer of PEG_{5000}-block-PLA (circle) or PEG_{5000}-block-PCL_{5000} (square) and for free β-lap (stars) in PBS (pH 7.4; open symbols) and acetate-buffered saline (pH 5.0; filled symbols) solutions at 37°C. Experimental data points for release from micelles as fitted by Higuchi's model and that for free β-*lap* as fitted by Fickian diffusion through a membrane are shown as solid lines.

methoxy-terminated PEG used in this study has a molecular weight of 5000 Da. Therefore, the thickness of micelle corona is estimated to be around 6.16 nm (40).

Results

Drug Loading and Micelle Characterization. The DOX loading content was quantified by UV-Vis analysis and was found to be 2.32% for PEG-*b*-PLA micelles and 3.80% for PEG-*b*-PCL micelles. β-Lap loading content was noticeably lower than that for DOX, as shown in Table 1. We note that, in both cases of β-lap and DOX, larger loading content is achieved for PEG-*b*-PCL micelles, possibly due to the larger hydrophobicity of PCL (as discussed below).

The micelle size with and without loaded drugs was studied by DLS and the results are listed in Table 1. The hydrodynamic diameter of PEG-*b*-PCL micelles without drug was found to be around 23.2 nm, while for PEG-*b*-PLA micelles, a smaller diameter around 17.4 nm was observed. The measured micelle sizes are comparable (PEG-*b*-PCL) or somewhat smaller (PEG-*b*-PLA) compared with other reported results (15–17, 24, 25). For instance, in a recent study by Liu *et al.* of similar molecular weight polymers, PEG-*b*-PCL micelles (24 nm) were found to be smaller than PEG-*b*-PLA micelles (66 nm) (16). The difference in micelle sizes could have originated from different micelle preparation techniques. Parameters such as mixing rate have been known to affect particle size by 3- to 4-fold (41), and the fast mixing resulting from the sonication mixer could be the cause of the small size of the micelles as compared with those made using a slow-mixing dialysis method.

Drug loading did not noticeably affect micelle size, as shown in Table 1. Student's *t* test analysis performed for the size comparison of loaded to unloaded micelles resulted in *P* values in the range of 0.24–0.70, indicating no significant difference in micelle sizes. Furthermore, comparing the sizes of all drug-loaded micelles (Table 2), one can observe that they are nearly the same: the largest difference between average micelle sizes is less than the smallest uncertainty range, and *t* test analysis of any given pair shows no significant variance. Based on these observations, we consider all drug-loaded micelles to be of the same size, defined by the average of the four values (i.e., $d = 21.08$ nm; standard deviation, ± 1.9 nm). Accordingly, the average core size calculated using Eq. 4 results in $a_o = 4.37$ nm, which we use to calculate the diffusion coefficients. It is also useful to estimate the average number of drug molecules per polymer micelle. Based on the drug-loading content and the estimated value of a_o, the number of drug molecules per micelle varies from about 12 for β-lap in PEG-*b*-PLA micelles to about 30 for DOX in PEG-*b*-PCL micelle. We note that the experimentally recorded data for drug release are the cumulative result of drug release from an ensemble of micelles, so that variation in number of molecules per micelle or in drug distribution inside micelles may not be important, as long as all drug molecules on average experience the same surrounding in their release pattern.

Release of β-Lap. The release profiles of β-lap from PEG-*b*-PLA and PEG-*b*-PCL micelles are shown in Figure 3. As is seen, the influence of pH on β-lap release is rather weak, with the deviations being within the experimental error range (based on results of three experiments). Since the solubility of β-lap is practically independent of pH, the only difference in release could come from changes in polymer matrix. Since we observe no appreciable effect of pH on drug release, this implies that no substantial change

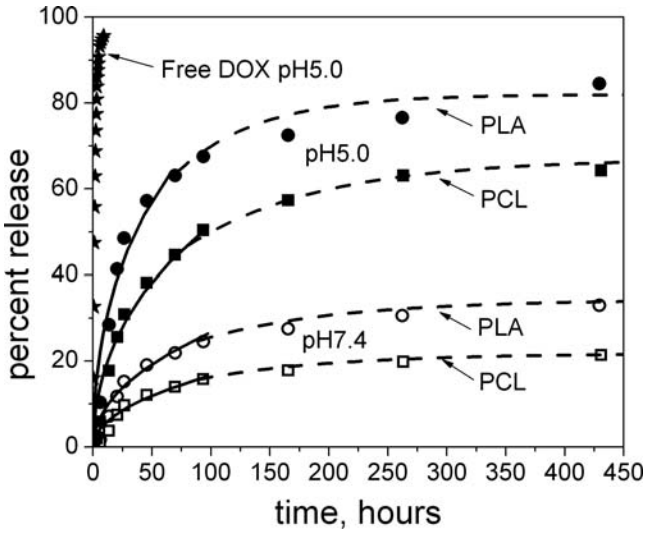


Figure 4. Cumulative release of DOX versus time from two different micelles composed of diblock copolymer of PEG_{5000}-block-PLA_{5000} (circle) or PEG_{5000}-block-PCL_{5000} (square) and for free DOX (stars) in PBS (pH 7.4; open symbols) and acetate-buffered saline (pH 5.0; filled symbols) solutions at 37°C. Experimental data points for short-term (up to 100 hrs) release from micelles are fitted by Higuchi's model (solid lines) and for long-term release are fitted by Fickian diffusion from a sphere (dashed lines).

in the micelle core occurs with a decrease of pH for either PEG-*b*-PLA or PEG-*b*-PCL micelles.

The release rate from PEG-*b*-PLA micelles is somewhat faster than for PEG-*b*-PCL micelles: 87% of β-lap was released from PEG-*b*-PLA micelles in 72 hrs, compared with 66% for PEG-*b*-PCL micelles during the same period of time. A similar trend was observed by Liu *et al*. (16) for ellipticine and PEG-*b*-PCL or PEG-*b*-PLA micelles of similar composition. Knowing the drug-loading concentration c_o (Table 2) and solubility of β-lap in the bulk liquid phase ($c_s = 0.038$ mg/ml [21]), we fit the diffusion of β-lap from these different polymeric media using Higuchi's model (34) for drug release from a sphere. Since the difference between the experimental data for pH 7.4 and pH 5.0 was very small for the same polymeric carriers, we used a single fit for both data sets. The results of fitting are shown as solid curves in Figure 3. The diffusion coefficient for β-lap release from PEG-*b*-PLA micelles was found to be 2.42×10^{-17}cm^2/sec, while that for β-lap release from PEG-*b*-PCL micelles was about 1.6-times smaller (1.49×10^{-17} cm^2/sec) (Table 2).

We note that in the control measurement of free β-lap transport across the dialysis membrane, the release occurs noticeably quicker. The corresponding diffusion coefficient obtained using a model of Fickian transport across a membrane (42) was 3.0×10^{-10} cm^2/sec (see Fig. 3 and supporting information), which is available in the on-line version, much larger than 2.42×10^{-17} cm^2/sec estimated for release from PEG-*b*-PLA micelles. Thus, the slowest step of the drug release is from the dense polymer core, which is well described by Higuchi's model (34). We were also able to obtain good fits to the β-lap micelle release data using a simple Fickian model of diffusion from a sphere (36). However, for the DOX release, discussed in the next section, the pH effects play an important role. As a result, we have chosen the Higuchi model (34) as a unifying approach for both β-lap and DOX-containing systems.

Short-Term Release of DOX. The release of DOX from PEG-*b*-PCL and PEG-*b*-PLA micelles at pH 7.4 and pH 5.0 is shown in Figure 4. There is a strong pH dependence of the release of DOX from different micelles: DOX release from the micelles at pH 5.0 is faster than at pH 7.4, and the total amount released at the longest investigated time is greater at pH 5.0 than at pH 7.4. For DOX-loaded PEG-*b*-PLA micelles, the amount of release at pH 5.0 is about 50% larger than that at pH 7.4, while for DOX-loaded PEG-*b*-PCL micelles, the difference is more than 40%. A similar effect of pH on DOX release has also been observed by Kataoka *et al*. using PEG-*b*-PBLA copolymer micelles (14). Similar to β-lap release, different core materials have an influence on DOX release, as PEG-*b*-PLA micelles have a faster release rate and a greater cumulative amount of drug released than PEG-*b*-PCL micelles.

The fitting of experimental data using Higuchi's model (34) (over relatively short time scales) is shown in Figure 4 as solid lines. For pH 7.4, using known drug-loading concentration, c_o (Table 2), and solubility of DOX in the bulk liquid phase ($c_s = 0.0625$ mg/ml [20]), fitting was performed by varying D/a_o^2. The following diffusion coefficients for DOX release were obtained (using the average value for micelle core radius, $a_o = 4.37$ nm [Table 2]): 1.82×10^{-18} cm^2/sec for PEG-*b*-PLA micelles and 1.13×10^{-18} cm^2/sec for PEG-*b*-PCL micelles (a factor of 1.6 difference). As shown by the release behavior of β-lap, the polymer in the micellar cores is unaffected by the pH. Therefore, the diffusion coefficient for DOX release from micelle cores is assumed to be independent of pH, and we fixed the diffusion coefficient to the value obtained for pH 7.4 and varied c_s/c_o to obtain the best match with experimental data for pH 5.0. The best-fit ratio of c_s/c_o is 1.57×10^{-2} for the DOX-loaded PEG-*b*-PLA micelle and 9.4×10^{-3} for DOX-loaded PEG-*b*-PCL micelle at pH 5.0 (Table 2). As is also shown in Table 2, for both types of DOX-loaded micelles, the ratio, c_s/c_o, increases by approximately a factor 12 when the pH is changed from 7.4 to 5.0. In the control measurement of free DOX transport across the same membrane, the release occurs noticeably quicker—100% of DOX releases from the membrane in less than 10 hrs (see Fig. 4 and supporting information). The corresponding diffusion coefficient obtained using the same transport across a membrane model discussed above was 1.3×10^{-9} cm^2/sec, much larger than the diffusion constant measured for micelles.

Long-Term Release of DOX. DOX release from different polymer micelles displays a noticeable deviation from Higuchi's model over longer periods of time. To

Table 3. Long-Term (over 100 Hrs) Release Characteristics of DOX from Micelles Composed of Diblock Copolymer of PEG_{5000}-*b*-PCL_{5000} or PEG_{5000}-*b*-PLA_{5000} at pH 5.0 and 7.4[a]

Drug	Micelle	pH	D/a_o^2 (1/sec)[b]	D (cm^2/sec)[b]	*p*[b]
DOX	PEG-*b*-PCL	5.0	$2.46 \times 10^{-7} \pm 0.05 \times 10^{-7}$	$4.70 \times 10^{-20} \pm 0.37 \times 10^{-20}$	0.67 ± 0.002
		7.4	$2.46 \times 10^{-7} \pm 0.28 \times 10^{-7}$	$4.70 \times 10^{-20} \pm 0.64 \times 10^{-20}$	0.218 ± 0.005
	PEG-*b*-PLA	5.0	$4.00 \times 10^{-7} \pm 0.49 \times 10^{-7}$	$7.65 \times 10^{-20} \pm 1.10 \times 10^{-20}$	0.82 ± 0.02
		7.4	$2.46 \times 10^{-7} \pm 0.43 \times 10^{-7}$	$4.70 \times 10^{-20} \pm 0.90 \times 10^{-20}$	0.34 ± 0.01

[a] D, diffusion coefficient of the drug in the core matrix; a_o, radius of the micelle core; *p*, fraction of drug released at "infinite time" (longest time of measurements); DOX, doxorubicin; PEG, polyethylene glycol; PCL, poly(ε-caprolactone); PLA, poly(D,L-lactide).
[b] Values are mean ± SD.

describe this time regime, we applied the long-time approximation for Fickian diffusion from a sphere (35, 36):

$$\frac{M(t)}{M(\infty)} = p\left(1 - \frac{6}{\pi^2}\exp\left(\frac{-\pi^2 D t}{a_o^2}\right)\right), \qquad (6)$$

where *p* is the fraction of drugs released at infinite time, which is approximated by the extrapolation of the fraction of drugs released at the longest times of the measurements (Table 3). These fits are shown as dashed lines in Figure 4. Of course, in reality, at even longer times, the additional mechanism of polymer decomposition will take over, resulting in release of the rest of the drug. Nonetheless, for this intermediate time scale, we obtained 7.65×10^{-20} and 4.7×10^{-20} cm^2/sec for the DOX diffusion coefficient from PEG-*b*-PLA micelles at pH 5.0 and pH 7.4, respectively, and 4.7×10^{-20} cm^2/sec for PEG-*b*-PCL micelles at both pH 5.0 and pH 7.4. As is seen, these diffusion coefficients are more than 20-times smaller than that for short-term DOX release (cf., Tables 2 and 3).

Discussion

Effects of pH on Drug Release. As we have discussed, there is hardly any influence of pH on β-lap release from either type of micelles. Since the solubility of β-lap is independent of pH, this suggests that there is little pH effect on the micelles themselves. However, DOX release is a totally different case, especially in the short-time regime. In the fit to DOX release by the Higuchi model (34) there is a 12-fold increase in c_s/c_o when the pH is lowered from 7.4 to 5.0. This is qualitatively consistent with the observations by Fritze *et al.*, who report a 6-fold increase of DOX solubility with a decrease of pH (from 0.0625 mg/ml at pH 7.4 to 0.37 mg/ml at pH 5.0 in phosphate buffer) (20). The solubility of protonated DOX (DOX-HCl) was reported to be even higher: 10–30 mg/ml (37, 38), leading to a solubility increase of more than 100-fold. As solubility of DOX in the buffer solution increases, its partition coefficient in polymeric media will decrease, leading to a greater amount of drug released. This is also reflected in the partition coefficient of DOX between the octanol/aqueous phases, decreasing from 1.20 at pH 7.4 to 0.23 at pH 5.0 (43). However, the effect of pH on the long-time DOX release is much smaller (see Table 3), suggesting that the dissolution-diffusion mechanism described by Higuchi is no longer operative.

Effects of Core Materials on Drug Release. From the release study, one can assume that the difference in the release rate from two different micelles could be partially caused by the drug-loading content, which is reflected in the ratio of c_s/c_o in Higuchi's model (34). Aside from the difference in loading contents, the physical properties of the core-forming polymer are also different, as PLA is less hydrophobic and more swellable than PCL. For instance, the water/air contact angle of PLA is reported to be in the range of 63°–69°, while the contact angle of PCL is in the range of 74°–93° (44–47). Sharp *et al.* have shown that water uptake by PLA is about 3% for 10 kDa molecular weight and increases with a decrease of molecular weight (48). The water uptake by PCL is very minor—it can be extrapolated to be not more than 1% (49). Another possible reason is the difference in physicochemical properties of PLA, an amorphous polymer, and PCL, a semicrystalline polymer (at least at large molecular weights). The crystalline structure of PCL could entrap drug inside the core, lowering the release rate of drug into the outside environment. The influences of the core materials on drug release could be observed for both β-lap and DOX. It is also interesting to note that the ratios of diffusion coefficients for DOX release from PEG-*b*-PLA and PEG-*b*-PCL (1.6) is essentially the same as that for β-lap release (1.6), even through the absolute values of the diffusion coefficient differ by about a factor of 10 (see Table 2). The latter is not surprising, considering that β-lap is a smaller molecule.

As discussed above, there is a noticeable deviation from Higuchi's model (34) for times longer than 100 hrs for DOX release from different polymer micelles, and Fickian diffusion (35, 36) is used for fitting long-term release instead. There have been several hypotheses and experimental verifications for the entrapment of DOX inside the micelle core, which delays its release from the micelle (14). As was discussed by Kataoka *et al.* (14), DOX has a tendency to form a chemically bonded dimer. Reverse-phase high-performance liquid chromatography analysis is used to study the dimer fraction, and the results show that, in our micelles, the amount of chemically bonded DOX dimer and higher-order complexes is around 10% for both PEG-*b*-PCL

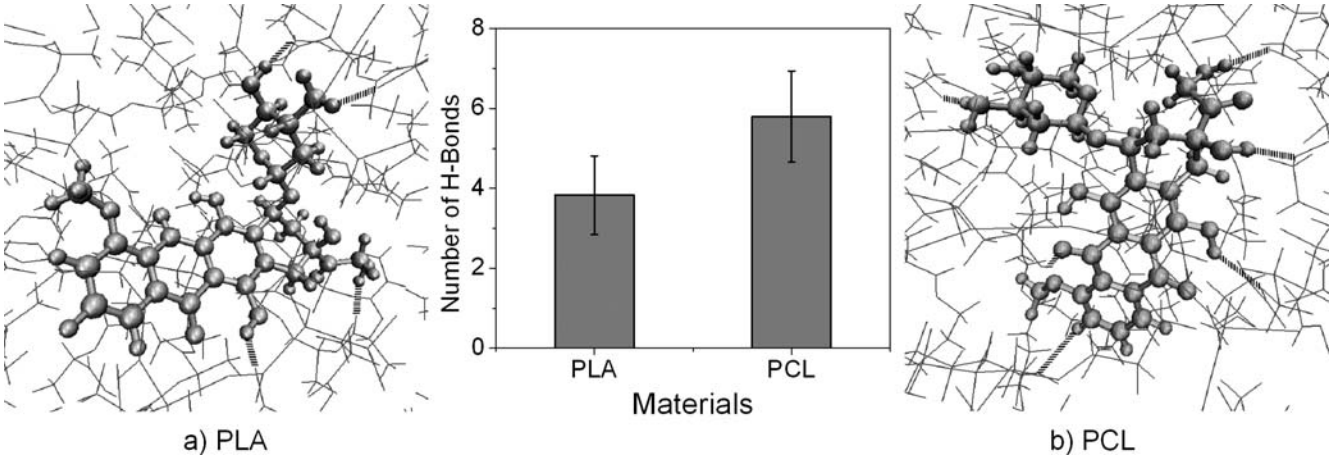


Figure 5. Typical snapshots from molecular dynamic simulations (using Impact 4 by Schrödinger) showing (A) DOX surrounded by PLA oligomers and (B) DOX surrounded by PCL oligomers. Oligomers are shown as lines and DOX is depicted using a "balls and sticks" representation. Hydrogen bonds are shown as dashed lines and their average number (based on 10 simulations each) is plotted in the middle plane.

and PEG-*b*-PLA micelles (see the supporting information available in the on-line version). However, in most cases, the limiting fraction of DOX release is far less than 90%. Therefore, it seems that, aside from the chemically bonded DOX dimer, there are other factors influencing the drug release results. Among the possible reasons is formation of physical aggregates of DOX (50–54) inside the micelles due to the lipophilic nature of the DOX (43). DOX is also known to adhere to dialysis tubing (55) and is capable of degradation in PBS buffer (56).

Another factor that may play some role in the entrapment of DOX is its specific interactions (hydrogen bonding) with the core-forming polymer. Hydrogen bonding between PCL and DOX has previously been detected experimentally using Fourier transform infrared spectroscopy (FTIR) (17). In this case, FTIR spectra of the PCL carbonyl band at 1735 cm^{-1} demonstrated formation of hydrogen bonds with entrapped DOX in a micelle core of similar molecular weight PCL ($M_n = 5000$). We have explored hydrogen bonding using molecular dynamic simulations (Impact 4.0 and Macromodel 8.5 by Schrödinger [57]) and demonstrated that, on average, five to six hydrogen bonds are formed between a single DOX molecule and surrounding PCL chains (Fig. 5). In contrast, when the core is PLA, the number of formed hydrogen bonds with DOX is somewhat smaller (3–4), which is consistent with a larger fraction of DOX release from PEG-*b*-PLA micelles. In both cases, there is a large excess of lactide monomers to DOX molecules (over 50-fold), indicating that different drug molecules are probably as fully hydrogen bonded as polymer conformation allows. For a larger DOX loading content, physical aggregation of DOX inside of micelles could occur (51, 53) in addition to hydrogen bonding between drug and polymer, making release even more complex.

Any of the above-mentioned factors could contribute to apparent slowing down of DOX release at longer time periods. At the moment, there is not sufficient experimental evidence to establish the dominant importance of any of these factors. More detailed comprehensive studies may be required to achieve this goal.

Conclusions. The encapsulation and release behaviors of two different drugs, DOX and β-lap, from two different micellar systems, PEG-*b*-PLA and PEG-*b*-PCL, with the same corona-forming polymers but different cores, were studied experimentally and by mathematical modeling. We found that core-forming material plays an important role in drug release due to the differences in their physicochemical properties and interactions with the drugs (15, 16, 58). The stronger interactions between hydrophobic drugs and PCL (a more hydrophobic, semicrystalline polymer) leads to a 1.6-fold slower release rate of both DOX and β-lap from PEG-*b*-PCL micelles compared with PLA (an amorphous, more swellable and hydrophilic polymer). The smaller of the two drugs, β-lap, has a more than 10-fold larger diffusion coefficient than DOX for both of the polymeric micelles considered. In contrast to pH-independent β-lap release from both PEG-*b*-PLA and PEG-*b*-PCL micelles, which demonstrated the absence of any pH effect on the core material, DOX release noticeably accelerated at lower pH, when it became protonated (14). This effect was well described by Higuchi's model, which accounts for the difference of solubility of drugs in different media *via* the ratio of c_s/c_o. At longer time periods, however, the release of DOX was better described by the Fickian diffusion model, which yielded a much smaller diffusion coefficient than the Higuchi model. The possible reasons for slowing down of DOX release at longer time periods include formation of chemical and physical DOX aggregates (50–54), specific interactions with the core-forming polymer (e.g., H-bond-

ing), adhesion of DOX on the dialysis membrane (55), and DOX degradation (56) during the course of measurements.

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