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Self-Assembled Lipid Cubic Phase and Cubosomes for the Delivery of Aspirin as a Model Drug

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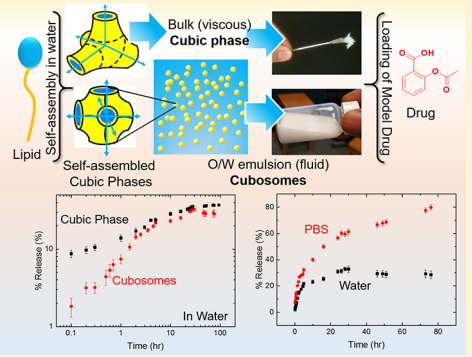
Supporting Information

ABSTRACT: Three-dimensionally organized lipid cubic self-assemblies and derived oil-in-water emulsions called "cubosomes" are attractive for various biotechnological applications due to their ability to be loaded with functional molecules and their associated sustained release properties. Here, we employed both of these lipid-based systems for the delivery of a model drug, aspirin, under comparable conditions. Studies were performed by varying drug-to-lipid ratio and the type of release medium, water and phosphate buffer saline (PBS). Release rates were determined using UV−vis spectroscopy, and small-angle X-ray scattering was used to confirm the type of self-assembled nanostructures formed in these lipid systems. The release from the bulk lipid cubic phase was sustained as compared to that of dispersed cubosomes, and the release in PBS was more efficient than in water. The tortuosity of the architecture, length of the diffusion pathway, type of nanostructure, and physicochemical interaction with the release media evidently contribute to these observations. This work is particularly important as it is the first report where both of these nanostructured lipid systems have been studied together under similar conditions. This work provides important insights into understanding and therefore controlling the release behavior of lipid-based drug nanocarriers.



INTRODUCTION

Lipids, generally composed of hydrophilic and hydrophobic molecular components (Figure 1a), tend to self-assemble in the presence of aqueous media. Self-assemblies can be as simple as spherical micelles and planar bilayers, or they can be quite elegant like hexagonal and cubic phases.[1,2] On the basis of their spatial organization, lipid cubic phases are divided into two types, bicontinuous and micellar.[3] Most common bicontinuous cubic phases (Figure 1b), defined by crystallographic space groups *Ia*3*d* (no. 230), *Pn*3*m* (no. 224), and *Im*3*m* (no. 229), are formed by draping a continuous lipid bilayer on gyroid (G), diamond (D), and primitive (P) type periodic minimal surfaces, respectively.[3,4] The term bicontinuous can be interpreted in two possible ways: (1) the continuity of two networks, the first made of a continuous bilayer and the second made of continuous waterways, and (2) the presence of two continuous aqueous channels separated by a single lipid bilayer.[5] Micellar cubic phases, on the other hand, are formed by an arrangement of discrete micelles in a cubic lattice.[3] A typical example of a micellar cubic phase is the *Fd*3*m* phase.[6]

Bicontinuous cubic phases are equipped with a range of structural features responsible for their applicability, for instance, (a) enormous surface area (400 m^2/g),[7] (b) large surface-to-volume ratio, (c) physicochemical ability to load hydrophilic molecules in the aqueous region, hydrophobic molecules in the lipid chain region, and amphiphilic molecules in bilayers,[8] (d) continuous trajectories for uninterrupted diffusion of loaded molecules,[5] (e) viscoelastic nature to provide stability and structural integrity for loaded molecules,[9] (f) highly organized porous network for nanoscale templating,[10] and (g) resemblance to biomembrane structures.[11] Further aspects include thermodynamic equilibrium[12] and robustness under given conditions as well as a high level of tunability. However, the preparation and handling of cubic phases are not simple protocols due to their inherently high viscosity (complex viscosity in the range of 10^4−10^5 Pa·s)[13] (Figure 1c). Two possible ways to overcome these hurdles are to utilize mechanical tools to handle cubic phases[14−16] or to disperse them into a fluid form[17,18] (Figure 1d,e). The latter provides additional benefits, for instance, improved surface-to-volume ratio,[19] availability of an enormous aqueous reservoir (up to 95−96% of the volume),[20,21] rather simple preparation protocols, low overall viscosity, and rather regular domains (monodispersed or low polydispersity particles).[19] Moreover,

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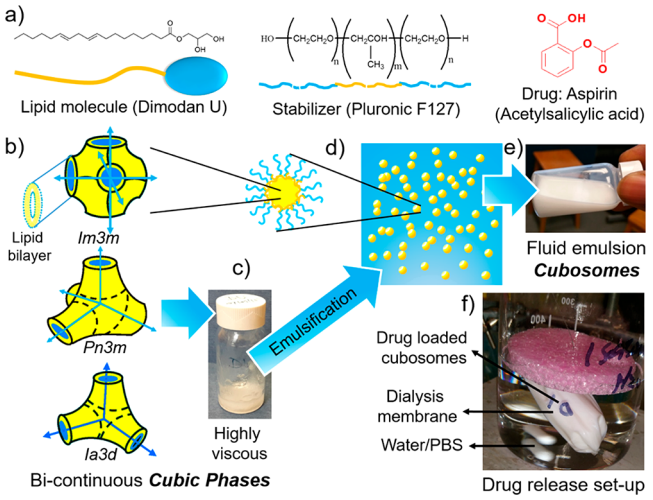

Figure 1. Schematic diagram depicting cubic phases, cubosomes, and release setup (not to scale). (a) Chemical structures of a lipid molecule, a surfactant stabilizer, and a drug. Blue and yellow indicates hydrophilic and hydrophobic parts/regions, respectively. (b) Bicontinuous cubic phases of *Im3m*, *Pn3m*, and *Ia3d* types with 6, 4, and 3 corresponding aqueous channels, respectively, as represented by arrows. (c) Highly viscous cubic phase (*Pn3m* in the current study) shown at the bottom of a 20 mL glass bottle. (d) Cubosome (left) displaying the *Im3m* phase as an internal self-assembly with surfactant molecules stabilizing the interface, and a dispersion of such cubosomes (right). (e) Cubosome dispersion, which is essentially a form of an oil-in-water emulsion, exhibiting a fluid and milky consistency. (f) Beaker containing water/PBS with dialysis membrane containing drug-loaded cubosomes. This represents the typical drug release setup employed in the current work.

dispersed lipid particles have great potential for engaging in targeted and tracked delivery applications.[21−29] Upon dispersion, the cubic phase is retained within the lipid particles; hence, the term "cubosomes" (Figure 1e) is used to describe them.[17] As compared to vesicles or liposomes, the bilayer area-to-particle volume ratio of cubosomes is higher;[19] in other words, cubosomes possess much larger hydrophobic volume fractions (corresponding values of 0.59 nm^3 as compared to 0.18 nm^3 for 100 nm sized particles).[21] This feature is very important for enhanced drug carrier capacity, especially for poorly water-soluble drugs.[19] In addition, cubosomes are much more robust and stable owing to their rather high viscosity that leads to a resistance to rupture.[19]

There are several reports demonstrating delivery applications using the bulk lipid cubic phase[30−39] and dispersed nanoparticles like cubosomes,[7,19,40−46] but there are hardly any records where both of these lipid systems have been studied together under comparable conditions. In this work, we examined the release properties of the bulk (nondispersed) lipid cubic phase and its dispersed form, i.e., cubosomes, for a model drug, aspirin (Figure 1a). Aspirin is an important drug exhibiting analgesic, antipyretic, and anti-inflammatory properties. Moreover, it was shown to increase the solubility of cholesterol plaques within the membrane[47] and increase its fluidity. However, aspirin has some predominant side effects including toxicity to the gastrointestinal tract and rapid conversion into less desired products.[48] Therefore, its encapsulation into an optimal delivery system would be largely beneficial.[48] We monitored the release of aspirin from the bulk lipid cubic phase and cubosomes in both water and PBS media (Figure 1f) using a UV−vis spectroscopic technique.

MATERIALS AND METHODS

Materials. A lipid, Dimodan U/J (DU), was generously provided by Danisco, A/S (Brabrand, Denmark). It is a distilled glyceride comprising 96% monoglycerides (Figure 1a), and the rest comprises diglycerides and free fatty acids. Two major monoglyceride components in DU are linoleate (62%) and oleate (25%). Hence, the hydrophobic part of DU contains mainly C_{18} chains (91%). The following chemicals/items were purchased from Sigma-Aldrich (Dorset, UK): triblock copolymer Pluronic F127 (PEO_{99}−PPO_{67}−PEO_{99}) (Figure 1a), used to stabilize lipid particles (cubosomes); aspirin (chemical name acetylsalicylic acid), which is a salicylate drug (Figure 1a) used mainly as an analgesic and antipyretic agent; phosphate buffered saline (PBS) tablets; and dialysis membrane sacks (with an average flat width of 35 mm and a MWCO of 12 000 Da). All chemicals were used as received without any further purification. DU was stored below 4 °C, whereas the other materials were kept at room temperature when not in use. Water used during the entire study was purified using Barnstead Nanopure system, Thermoscientific (USA).

Standard Calibration Curves of the Drug in Water and PBS. Aspirin (50 mg) was dissolved separately in 10 mL of distilled deionized water (henceforth, "water") and 10 mL of 0.01 M PBS buffer (one tablet dissolved in 200 mL of deionized water yields 0.01 M phosphate buffer at pH ∼ 7.4; henceforth, "PBS") in 100 mL volumetric flasks. The mixtures were sonicated for 5 min (Sonics & Materials Vibra-Cell VCX750, Jencons, UK) to ensure drug dissolution. Finally, the corresponding volumes were brought up to the mark with water and PBS to make 0.5 mg/mL stock solutions. Standard solutions were prepared by appropriate dilutions of the stock solutions. The absorption spectra of aspirin solutions were determined in the ultraviolet visible (UV) range of 200−800 nm with the characteristic λ_{max} at 276 nm (UV-1600PC, VWR, UK). At least 10 dilutions, in the concentration range of 0.1−100 μg/mL, were utilized to establish standard calibration curves at the λ_{max} (see Supporting Information Figure S1). All measurements were performed in triplicates at room temperature (∼25 °C).

Preparation and Loading of the Drug in the Cubic Phase (Bulk Lipid Phase). The bulk lipid phase was prepared by melting 2 g of lipid (DU) in a 20 mL glass vial followed by the addition of an equal amount of water (by weight), yielding a known *Pn3m* cubic phase[49] (Figure 1c). The drug-loaded bulk phase was prepared as follows: A mixture of the appropriate drug concentration and 1 g of molten DU was stirred (using a magnetic stirrer bar) for 30 min at 50 °C followed by the addition of 1 g of water. During the release experiments, a further 9 g of water was added to 1 g of the cubic phase in order to maintain concentrations comparable to those of the cubosomes solution. The hydrated phase was physically mixed with a spatula and allowed to stand overnight at room temperature.

Preparation and Loading of the Drug in Cubosomes (Dispersed Lipid Particles). The nanostructured lipid particle dispersions, i.e., cubosome solutions, were prepared according to a published method, although slightly different parameters and compositions were used.[50] Molten lipid (DU; 500 mg) was added to a 20 mL glass vial and topped with 9.5 g of F127 stabilizer solution (already prepared by dissolving 0.5% Pluronic F127 powder in 100 g of water). The resulting (10 g) mixture was ultrasonicated (Sonics & Materials Vibra-Cell VCX750, Jencons, UK) for 5 min with a continuous pulse using 35% of the maximum power. The sample (milky fluid emulsion, Figure 1e) became hot due to ultrasonication and was left to cool to room temperature before further usage. Drug-loaded samples were prepared by stirring the appropriate weight of the drug in the above cubosome solution for about 20 min at 50 °C.

Drug−Lipid Mixtures and a Release Setup. A drug-loaded bulk cubic phase mixture (10 g, containing 1 g of lipid cubic phase and 9 g of water) and 10 g of dispersed cubosomes were loaded with a range of drug concentrations to obtain final samples at 2, 4, 6, and 10 wt %/wt drug/lipid. The above samples were carefully transferred into dialysis membrane sacks (which were prepared by heating in pure water for 10 min at 80 °C). Both ends of the membrane sack were tightly tied to avoid sample leakage. Finally, the membrane sacks were individually immersed into 200 mL of either water or PBS in 500 mL beakers. A 3

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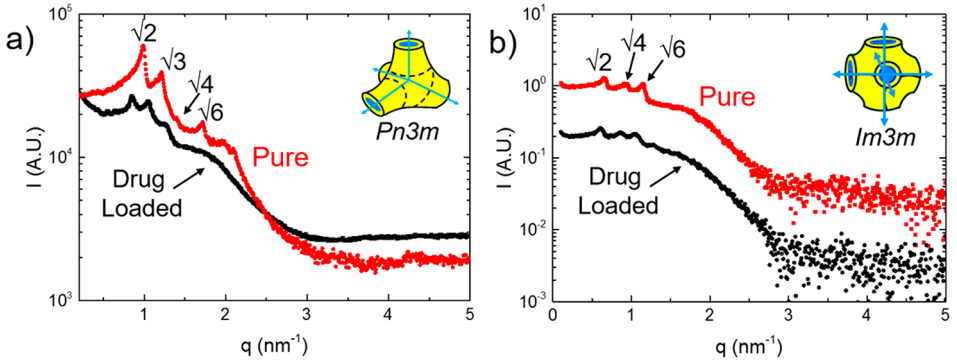

Figure 2. Detection of the type of lipid self-assembly using SAXS. (a) Observation of the *Pn*3*m* cubic phase in pure and drug-loaded (10% drug) bulk lipid systems. (b) Observation of the *Im*3*m* cubic phase in pure and drug-loaded (10% drug) dispersed cubosome systems. Characteristic Bragg diffractions for the *Pn*3*m* ($\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$) cubic phase and the *Im*3*m* ($\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$) cubic phase are shown near the corresponding peaks. Aqueous channels are indicated by arrows.

mL solution from the aforementioned release setup (beaker) was collected after different lengths of time including t = 0 min, and the UV−vis spectroscopic data was measured at a λ_{max} of 276 nm. After recording the measurements, the solutions were poured back into their original reservoir to maintain accumulative release conditions. The studied drug concentrations were well within sink conditions.[51]

Entrapment Efficiency (%) and Drug Loading (%) in Cubosomes and Bulk Cubic Phase. Drug (1 g, 2%) loaded dispersed cubosomes were added to a 1.5 mL Eppendorf tube and centrifuged at 13 200 rpm for 10 min (Spectrafuge 24D from Jencons Pls). The fluid phase was transferred into an empty Eppendorf tube and centrifuged for a further 10 min. The majority of the cubosome particles were removed by this method; however, the emulsion retained its opacity. The remaining lipid was separated using an Amicon Ultra-0.5 centrifugal filter unit with Ultracel-3 membranes (NMWL 3 kDa, Millipore, USA) with 20 min of centrifugation at 13 200 rpm. The separated aqueous phase was diluted with water, and the absorbance at 276 nm was measured using UV−vis spectroscopy. The entrapment efficiency (EE) and drug loading (DL) were determined using following formulas.[52]

$$EE = \left(1 - \frac{C_U}{C_T}\right) \times 100 \quad (1)$$

$$DL = \left(\frac{C_T - C_U}{C_L}\right) \times 100 \quad (2)$$

where C_U is the concentration of nonentrapped drug (free unloaded drug), C_T is the concentration of drug added to the cubosome dispersion, and C_L is the total lipid content. The EE and DL values for the other cubosome dispersions (with 4, 6, and 10% drug) were determined in the same manner as descried above. Excess water (aqueous phase) from the drug-loaded bulk cubic phase system was separated simply by decanting into empty cuvettes followed by recording the absorbance at 276 nm. The EE and DL values for 2, 4, 6 and 10% drug-loaded bulk cubic phase samples were determined subsequently using the above formulas (eqs 1 and 2).

Particle Size Analysis of Cubosome Dispersions. The mean particle size and size distribution of cubosome dispersions were measured using a Zetasizer Nano ZS instrument (Malvern Instruments, UK) operated at 25 °C.

Detecting the Type of Lipid Nanostructure Using Small Angle X-ray Scattering. Small angle X-ray scattering (SAXS) was used to detect the type of lipid nanostructure of the bulk and dispersed lipid systems (Figure 2). A SAXSpace instrument (Anton Paar, Graz, Austria) at University of Leeds was employed for this purpose. Details of the instrument and the measurements were published previously.[53] The instrument is based on a Cu anode operating at 40 kV and 50 mA. Samples were measured using a capillary sample holder controlled at 25 ± 0.1 °C. Typical exposure times of 300 s were sufficient to obtain patterns with well-resolved peaks (Figure 2).

RESULTS AND DISCUSSION

Nanostructural Characterization and Drug Loading Capacity of Bulk Cubic Phase and Cubosomes. The type of nanostructural self-assembly was determined using SAXS for drug-loaded and native lipid systems. Bulk DU formed a *Pn*3*m* type bicontinuous cubic phase in excess water,[49] which was retained upon the addition of 10% drug (Figure 2a). In the case of dispersed lipid systems, the addition of stabilizer molecules usually caused a phase transition into the *Im*3*m* type bicontinuous cubic phase[20,54,55] (Figure 2b).

The ultrasonic dispersion process created discrete lipid particles with an internal nanostructure of cubic phase. These cubosomes exhibited a submicrometer size, as verified by particle size analysis (for the plot, see Supporting Information Figure S2). The particle size values for native and drug-loaded cubosomes are listed in Table 1.

Table 1. Particle Size and Polydispersity Indices of Native and Drug-Loaded Cubosomes Measured by Dynamic Light Scattering with a Zetasizer Nano ZS

drug in dispersion (wt %/wt of lipid)	average particle size (nm)	polydispersity index
0	194 ± 4	0.210
2	184 ± 3	0.192
4	183 ± 3	0.207
6	182 ± 2	0.205
10	184 ± 1	0.184

The EE of the bulk lipid cubic phase in excess water and that of a cubosome dispersion were calculated using eq 1 as shown in Table 2. Similarly, drug loading in the lipid cubic phase and in cubosome particles was calculated using eq 2 and is shown in Table 2. The EE and DL values for the bulk cubic phase were higher compared to those of the cubosome system. The low DL values demonstrate that the drug is preferably located in the aqueous phase rather than in lipid structures. Upon encapsulation in lipid systems and subsequent dehydration, the structural features of the drug (aspirin) are generally protected, as reported in our previous work.[56] However, the lipid systems employed in the current study were always under excess water conditions, permitting drug encapsulation in both the lipid and aqueous regions (Table 2).

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Table 2. Entrapment Efficiency (EE) and Drug Loading (DL) in the Bulk Lipid Phase and Dispersed Cubosomes

drug loading (wt %/wt of lipid)	EE bulk cubic phase in excess water (%)	EE cubosome dispersion (%)	DL cubic phase (%)	DL cubosomes (%)
2	84.1	61.9	0.39	0.25
4	98.7	67.6	0.91	0.54
6	98.5	71.3	1.36	0.86
10	96.6	71.6	2.22	1.43

Drug Release from Bulk Cubic Phase. Referring to the standard calibration curves obtained by dissolving various concentrations of the drug in water and PBS (Supporting Information Figure S1), the measured absorbance values at λ_{max} of 276 nm were translated into percentage (%) release values. These values were then plotted against time (recorded in hours) for release from the bulk cubic phase in water (Figure 3) and PBS (Figure 4).

The curves in Figures 3 and 4 essentially followed typical drug release kinetics. Their logarithmic plots fitted well (with correlation coefficient R^2 values > 0.96) with the characteristic Korsmeyer–Peppas equation (eq 3);[57,58] for representative fits, see Supporting Information Figure S3.

$$M_t/M_\infty = Kt^n \quad (3)$$

where M_t is the amount of drug released at time t, M_∞ is the total amount of drug in the formulation, K is a kinetic constant, n is the exponent, characteristic of the release mechanism, and t is the release time in hours.

The release of the drug in water became insignificant after about 30 h (Figure 3a), but it was gradual and well-detectable up to about 96 h using PBS as the release medium (Figure 4a). The slopes of the fit lines, i.e., release rates in %/h, follow a linear trend except for the 10% drug sample (Figures 3b and 4b). The corresponding slopes of these lines were comparable: 0.05198% wt%/h for release in water (Figure 3b) and 0.04701% wt%/h for release in PBS (Figure 4b). Both release rates are dependent on the drug concentration and follow first-order kinetics.

Drug Release from Dispersed Cubosomes. The release trends from cubosomes (Figure 5) followed similar release patterns as those from the bulk cubic phase (Figures 3 and 4). The release in water saturated after about 30 h, whereas it continued gradually in PBS.

However, the release from cubosomes in water (Figure 5a) appears to be less dependent on the drug concentration, as evident from the closely positioned release curves. Nevertheless, the release curves on a log–log scale, both in water and in PBS, were fitted using the Korsmeyer–Peppas equation (eq 3)[57] with typical R^2 values > 0.93.

Sustained Drug Release from the Bulk Cubic Phase Compared to Cubosomes. Comparing the release rates from cubic phases and cubosomes (Figure 6), it is clear that the release from cubosomes is faster than the release from bulk cubic phases. The release of 2% drug from the bulk cubic phase was found to be sustained 3-fold (0.233%/h) better than the release from cubosomes (0.658%/h) when measured in water, whereas it was sustained twice as much from the bulk cubic phase (0.190%/h) than from cubosomes (0.402%/h) in PBS. The same trend was also observed for the release rate values of 4% drug in water (0.283%/h for the bulk cubic phase and 0.637%/h for cubosomes) and in PBS (0.282%/h for the bulk cubic phase and 0.407%/h for cubosomes). The release rates for 6 and 10% drug from cubosomes were also faster than the release rates from bulk cubic phases, both in water and PBS.

The goodness of fit of the Korsmeyer–Peppas equation to almost all curves in both lipid systems helps to understand their underlying release mechanisms. Analysis of the exponent n (eq 3) suggests that the release mechanism is Fickian diffusion when n is less than or equal to 0.45 ($n \leq 0.45$). The release from the bulk cubic phase, both in water and in PBS, followed this mechanism. Apparent Fickian diffusion is known to contribute to the sustained release of small molecules from bulk cubic phases.[30,31,35−39] This can be easily attributed to the highly tortuous porous morphology of cubic phases. In order to release from the bulk cubic phase, the drug molecule has to navigate through a lengthy aqueous network assembled by the long and continuous lipid bilayer architecture. On the contrary, the release from cubosomes is rapid and less sustained (Figure 6). This is due to their smaller (<200 nm, Table 1)[53] particle size, which is correlated to smaller length scales for drug transport, and to their surface area being too high, leading to burst release kinetics.[59] The release from cubosomes in water displayed an n value below 0.45, but for the release in PBS, the n value was greater than 0.45 (n = 0.63), indicating anomalous or non-Fickian diffusion kinetics.[57,58]

A few recent reports have demonstrated that the release rates from lipid systems can be controlled by fine tuning the nanostructure type and the dimensions of the self-assembled

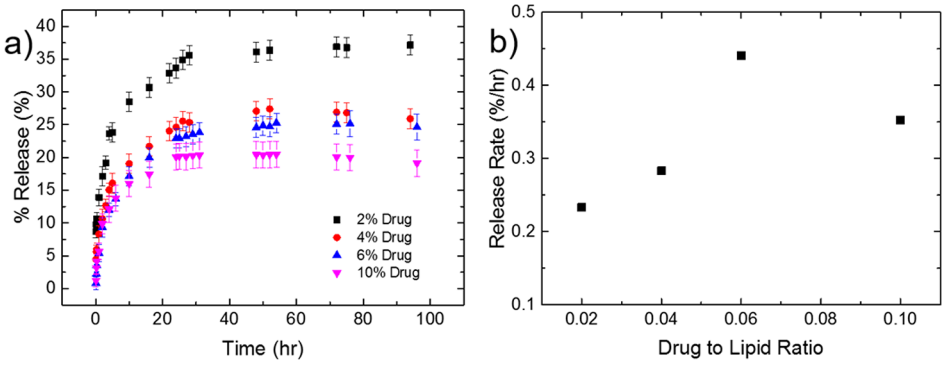

Figure 3. Drug release from the bulk cubic phase in water. (a) Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) shown against time in hours for 2, 4, 6 and 10% drug. (b) Curves in (a) were fitted with the Korsmeyer–Peppas equation to obtain the release rates (%/h), which are plotted against the drug-to-lipid ratio.

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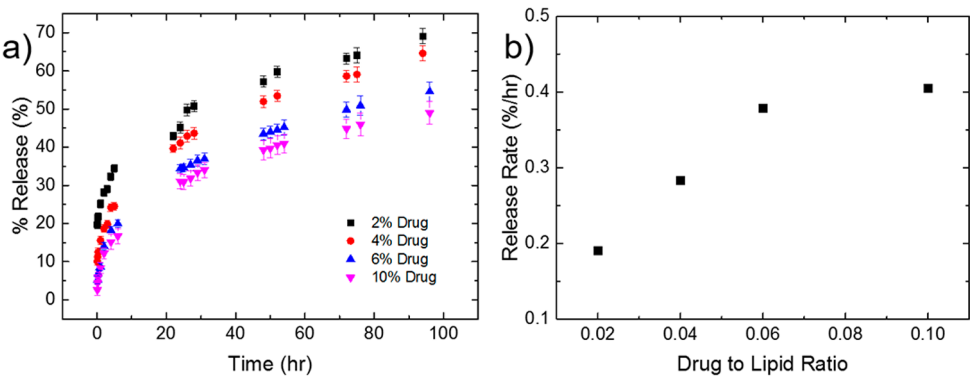

Figure 4. Drug release from the bulk cubic phase in PBS. (a) Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) shown against time in hours for 2, 4, 6 and 10% drug. (b) Curves in (a) were fitted with the Korsmeyer−Peppas equation to obtain the release rates (%/h), which are plotted against the drug-to-lipid ratio.

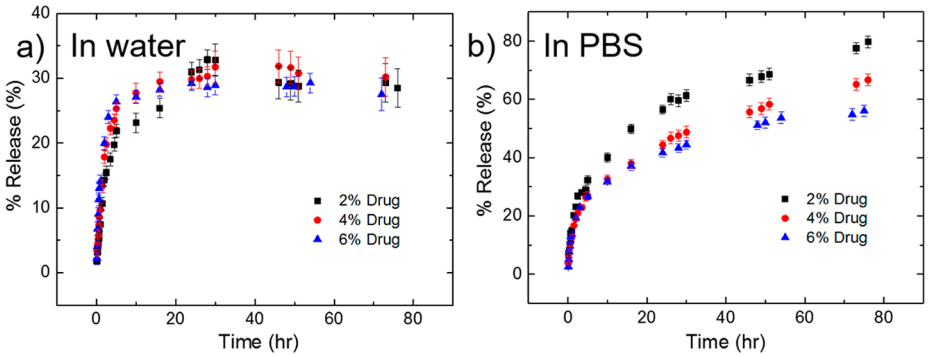

Figure 5. Drug release from dispersed cubosomes in (a) water and (b) PBS. Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) plotted against time in hours for 2, 4, 6 and 10% drug/lipid.

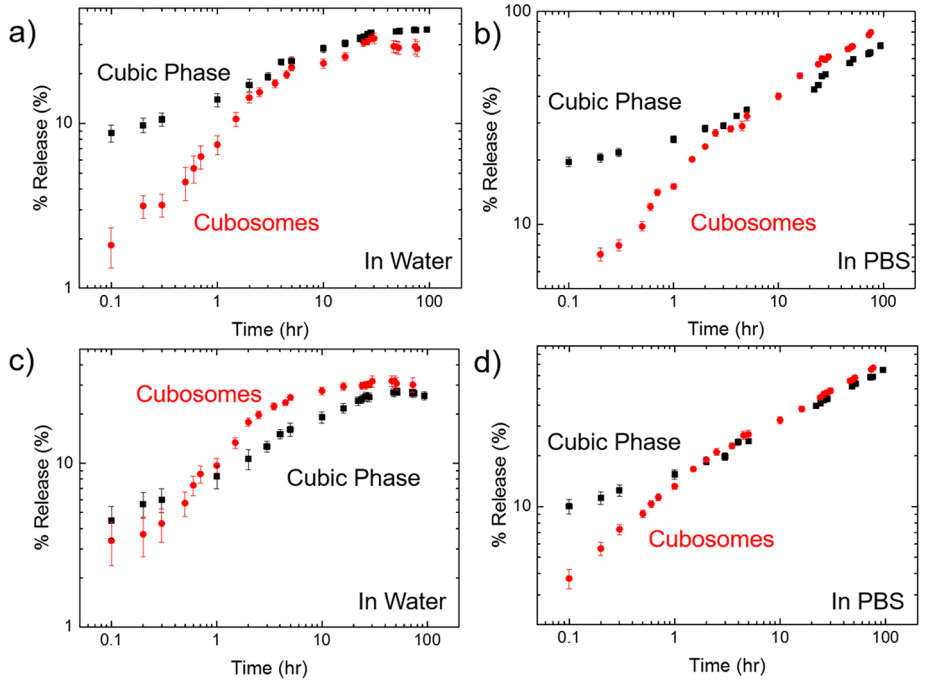

Figure 6. Comparisons of drug release from the bulk cubic phase and cubosomes. Percent (%) release for 2% drug in (a) water and (b) PBS and 4% drug in (c) water and (d) PBS are shown over time in hours.

phases.[30−32,60−62] For instance, if the nanostructure is a hexagonal phase, the release can be better controlled in comparison to that of open structured cubic nanostructures.[34,63] Moreover, the release could be promoted via stimuli-responsive (pH, light, or temperature induced) triggers.[63−65] We have recently demonstrated that it is possible

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Table 3. Nanoscale Dimensions of Lipid Cubic Phases Formed in the Bulk System and Dispersed Systems before and after Drug Loading[a]

physical form of lipid system	concentration of drug wt %/lipid wt	type of self-assembled cubic phase	no. of aqueous channels	lattice parameter (a; Å)	diameter of aqueous channel (dw; Å)
bulk cubic phase	0	*Pn*3*m*	4	89.5	37.9
bulk cubic phase	10	*Pn*3*m*	4	103.2	48.7
dispersed cubosomes	0	*Im*3*m*	6	135.6	50.9
dispersed cubosomes	10	*Im*3*m*	6	146.7	57.8

[a]Lattice parameters and diameters of aqueous channels were calculated according to the formulae listed in ref 67.

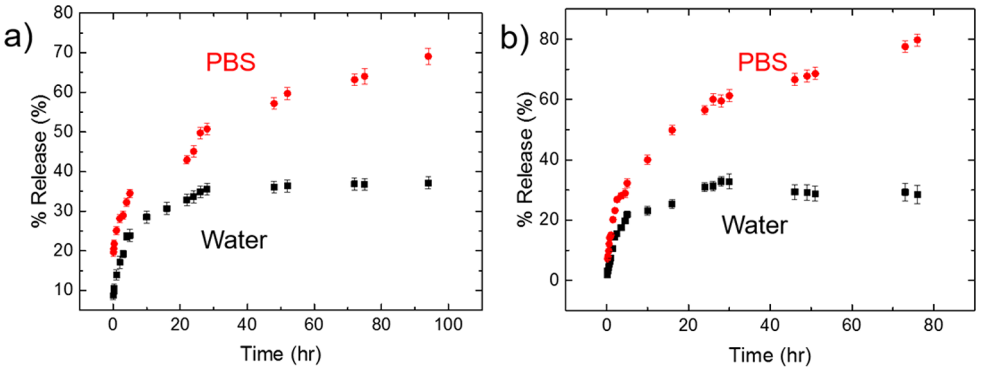

Figure 7. Drug release in PBS compared to water. Percent (%) drug release in water (solid squares) and in PBS (solid circles) for 2% drug/lipid is shown for the (a) bulk cubic phase and (b) cubosome system. Note that the representative data plots for 2% drug/lipid samples are shown here; for remaining data plots (4, 6, and 10% drug/lipid), see Supporting Information Figure S4.

to sustain the release from cubosomes by encapsulating them into hydrogel films.[56,66] The release, in this case, is believed to occur in multiple steps involving hydration of the hydrogel film, swelling and subsequent erosion of the hydrogel, opening of the aqueous channels of the cubosomes, burst release, and finally dissolution of the hydrogel matrix.[56]

The nanoscale design and dimensions of cubic self-assemblies further influence their release kinetics as follows. The lipid used in this work forms a *Pn*3*m* type cubic phase in pure water[49] (Figure 2a). This phase exhibits four aqueous channels (meeting at a tetrahedral angle), whereas the internal self-assembly of cubosomes is found to be the *Im*3*m* phase (Figure 2b) consisting of six aqueous channels (meeting at right angles). Moreover, the aqueous channels of the *Im*3*m* phase are usually larger than those of the *Pn*3*m* phase for the same lipid system; see Table 3. Largely hydrophilic block copolymer stabilizers, similar to F127 used in this work, are known to alter the average molecular shape of cubic phase-forming lipids;[20,54] the most common result is the formation of the *Im*3*m* phase in the internal cores of dispersed cubosomes.[55]

The encapsulation of a drug further elevates the diameter of the aqueous channels by about 22% in the bulk cubic phase and 12% in cubosomes (Table 3). Thus, the number of aqueous channels and their size are greater in the cubic phase (*Im*3*m*) of cubosomes as compared to those of the bulk cubic phase (*Pn*3*m*), which theoretically contributes to the rather rapid release from cubosomes.

More Efficient Release in PBS than in Water. Drug release from all samples, in the bulk cubic phases and cubosomes, in PBS was far more efficient (more than double in some cases) than the release in water (Figure 7 and Table 4). PBS (pH ∼ 7.4) is generally considered to represent physiological conditions, where drug release kinetics is usually different than under ambient and pure conditions.

Table 4. Percent Release at 24 h from Both Lipid Systems in Water and PBS

drug (wt %/wt of lipid)	bulk cubic phase in water (%)	bulk cubic phase in PBS (%)	cubosomes in water (%)	cubosomes in PBS (%)
2	33.65	45.12	30.93	56.53
4	24.62	41.11	29.47	44.39
6	22.91	34.39	29.16	41.67
10	20.09	31.05	18.23	38.96

The main advantages of employing PBS for release studies include the retention of a constant pH, prevention of denaturation and/or conformational changes, resemblance to body conditions (isotonic), and nontoxicity to cells.[68] However, biological buffers and their associated pH affect membrane properties.[68,69] It was envisaged that the buffer molecules would interact with hydrophilic lipid head groups and intercalate within the headgroup region, facilitating a reduction of the membrane bending modulus and an increase in the number of membrane fluctuations.[69] The overall effect of these events is revealed in the form of swelling[68] and/or softening of lipid membranes.[69] This could be directly linked to the continued and efficient release of drug from lipid carriers in PBS in contrast to the low and halted release in pure water (Figure 7 and Table 4).

The release (%) measured after 24 h for the bulk cubic phase and cubosomes in both media, i.e., water and PBS, always decreased with an increase in the drug/lipid ratio (Table 4). This again confirms that the release rate depends on the drug concentration, thus following first-order release kinetics.

CONCLUSIONS AND PERSPECTIVES

In this work, we compared the release properties of two different lipid nanocarrier systems. The bulk lipid cubic phase and cubosomes were studied under similar drug-to-lipid ratios

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and volumes of release media. Drug release in both lipid systems followed a mechanism described by the Korsmeyer–Peppas equation, revealing Fickian diffusion through the porous matrix.[57,58] However, the diffusion from cubosomes in PBS was anomalous, involving either or a combination of non-Fickian diffusion and erosion.[57,58] The latter may correspond to the interaction of PBS buffer molecules with lipid bilayer structures[68,69] that are enclosed by small cubosome particles. This can be verified by the efficient and rapid release in PBS compared to the almost stopped release in water (Figure 7).

The release from bulk lipid systems was sustained with respect to the dispersed colloidal cubosomes for apparent reasons. Although both lipid systems exhibit self-assembled cubic nanostructures with highly tortuous porous networks, the effective length scales experienced by the drug molecules differ enormously. Cubosome particles are small in size with a rather high interfacial area, leading to a burst release, whereas the longer length scales of the bulk cubic phase enable prolonged drug release. Moreover, the wider and higher number of aqueous channels of the *Im3m* cubic phase contribute to the enhanced release from cubosomes. At this stage, we cannot comment on the role of the overall high viscosity of bulk cubic phases in the release kinetics, and we leave this for further in-depth studies. This study sheds light on an important aspect of comparing two emerging drug delivery systems based on the nanoscale self-assembly of lipid molecules in aqueous media. The results obtained are potentially useful for predicting the design of and optimizing these nanocarrier systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b02486.

Standard calibration curve for aspirin; particle size distribution of native and drug-loaded cubosomes; release data of 4% drug in the bulk cubic phase; percent drug release (PDF)

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Notes

The authors declare no competing financial interest.

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