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

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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 associated sustained release properties. Here we employed both 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 while small angle X-ray scattering confirmed the type of self-assembled nanostructures in lipid systems. The release from bulk lipid cubic phase was sustained as compared to the dispersed cubosomes while the release in PBS was efficient than in water. Highly tortuous 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 the nanostructured lipid systems were studied together under similar conditions. The work provides important insights in understanding, and therefore controlling the release behaviour of lipid-based drug nanocarriers.

Introduction

Lipids, generally composed of hydrophilic and hydrophobic molecular components (Figure 1a), tend to self-assemble in 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]. Based on the spatial organisation, lipid cubic phases are divided into two types[3], bicontinuous and micellar[3]. Most common bicontinuous cubic phases (Figure 1b), defined by crystallographic space groups *Ia3d* (no. 230), *Pn3m* (no. 224) and *Im3m* (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 '*bi*continuous' can be interpreted in two possible ways, 1) continuity of *two* networks- first made of a continuous bilayer and second made of continuous waterways, and 2) 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]. Typical example of a micellar cubic phase is *Fd3m* phase[6].

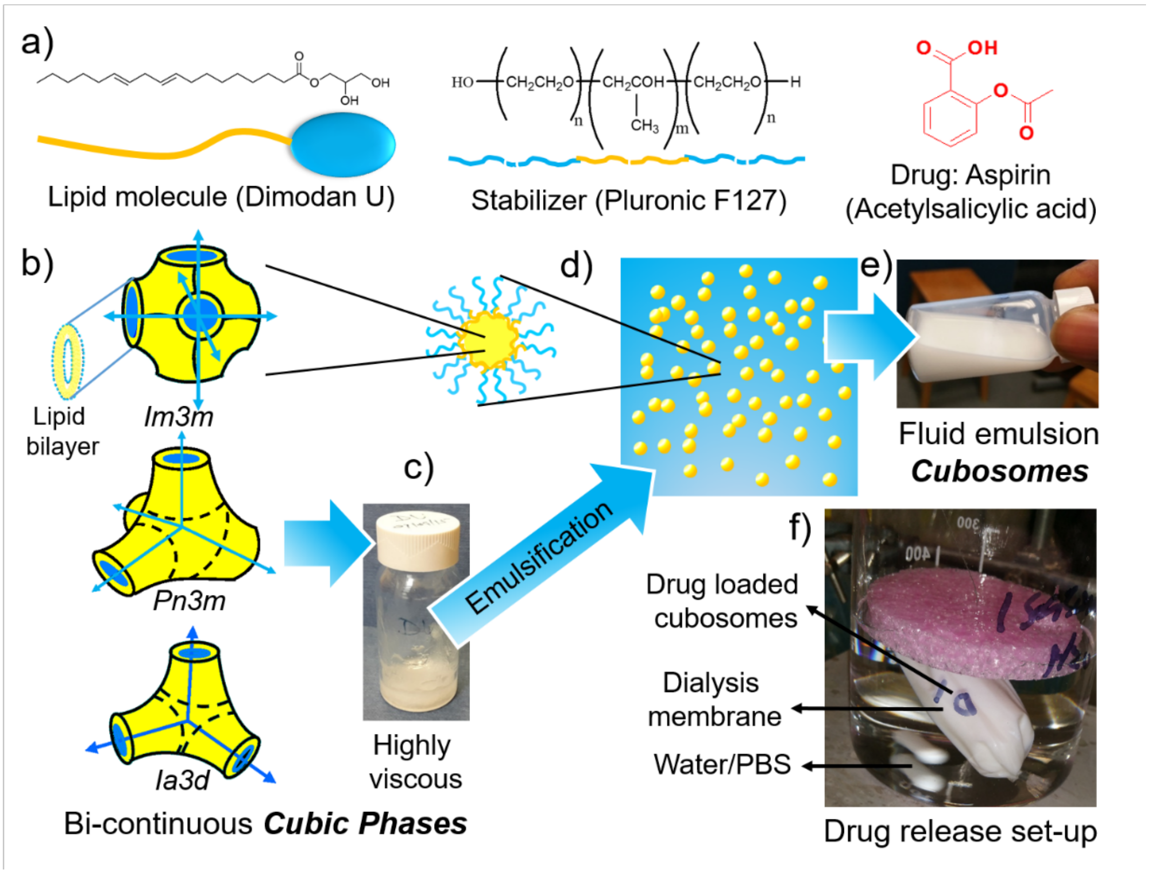

Figure 1: Schematic diagram depicting cubic phases, cubosomes and release set-up (not on scale): a) Chemical structures of a lipid molecule, a surfactant stabilizer and a drug. Blue and yellow colour shades code for hydrophilic and hydrophobic parts/regions, respectively. b)

Bicontinuous cubic phases of *Im3m*, *Pn3m* and *Ia3d* types with corresponding 6, 4 and 3 aqueous channels as represented by arrows. c) Highly viscous cubic phase (*Pn3m* in the current study) is shown at the bottom of 20 ml glass bottle. d) Graphics of cubosome on the left displays *Im3m* phase as an internal self-assembly while the surfactant molecules stabilize the interface; dispersion of such cubosomes is shown on the right. e) Cubosome dispersion is essentially a form of an oil-in-water emulsion, which exhibits fluid and milky consistency. f) A beaker containing water/PBS with a dialysis membrane holding drug-loaded cubosomes. It represents a typical drug release set-up employed in the current work.

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 aqueous region, hydrophobic molecules in lipid chain region and amphiphilic molecules in bilayers[8] d) continuous trajectories for uninterrupted diffusion of loaded molecules[5] e) viscoelastic nature for the stability and structural integrity of loaded molecules[9] f) highly organized porous network for nanoscale templating[10] and g) resemblance with biomembrane structures[11]. Thermodynamic equilibrium[12] and robustness under given conditions, and high level of tunability are further aspects. However, the preparation and the handling of cubic phases are not simple protocols due to inherent 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 means provides additional benefits, for instance, improved surface to volume ratio[19], availability of 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 poly-dispersity particles)[19]. Moreover, 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 cubosomes exhibit higher bilayer area to particle volume ratios[19], in other words, the 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 of poorly water-soluble drugs[19]. In addition, the cubosomes are much more robust and stable owing to rather high viscous resistance to rupture[19].

There are several reports demonstrating the delivery applications of bulk lipid cubic phase[30-39] as well as dispersed nanoparticles like cubosomes [7, 19, 40-46] but there is hardly any record where both of the lipid systems are studied together under comparable conditions. In this work, we examined the release properties of bulk (non-dispersed) lipid cubic phase and it's dispersed form i.e. cubosomes for a model drug called 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 the toxicity of the gastrointestinal (GI) tract and rapid conversion into less desired products[48]. Therefore, its encapsulation into optimal delivery system would be largely beneficial[48]. We monitored the release of aspirin from bulk lipid cubic phase and cubosomes, in water and PBS media (Figure 1f), using 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 are diglycerides and free fatty acids. Two major monoglyceride components in DU are linoleate (62%) and oleate (25%). Hence the hydrophobic part of DU mainly contains 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); it is a salicylate drug (Figure 1a) used mainly as analgesic and antipyretic agent, phosphate buffer saline (PBS) tablets, and dialysis membrane sacks (with an average flat width of 35 mm, MWCO 12,000 Da). All the 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, Thermoscientific (USA).

Standard calibration curves of a drug in water and PBS

50 mg of aspirin was dissolved separately in 10 ml *distilled deionised water* (*now on referred as 'water'*) and 10 ml 0.01 M PBS buffer (one tablet dissolved in 200 ml of deionized water yields *0.01 M phosphate buffer with pH ~7.4, now on referred as 'PBS'*) in 100 ml volumetric

flasks. The mixtures were sonicated for 5 min (Sonics & Materials Vibra-Cell VCX750, Jencons, UK) to ensure the drug dissolution. Finally, the corresponding volumes were made up to the mark with water and PBS to make 0.5 mg/ml stock solutions. Standard solutions were prepared by appropriate dilutions of above stock solutions. The absorption spectra of aspirin solutions were determined in the ultra-violet 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 range of 0.1 to 100 µg/ml concentrations, 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 a drug in the cubic phase (bulk lipid phase)

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

Preparation and loading of a drug in cubosomes (dispersed lipid particles)

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

Drug lipid mixtures and a release set-up

10 g of drug-loaded bulk cubic phase mixture (containing 1 g lipid cubic phase and 9 g water) and 10 g dispersed cubosomes were loaded with a range of drug concentrations to obtain final samples of 2 %, 4 %, 6% and 10% wt/wt drug/lipid. Above samples were carefully transferred

into dialysis membrane sacs (which were prepared by heating in pure water for 10 min at 80 °C). Both ends of membrane sacs were tightly tied to avoid sample leakage. Finally the membrane sacs were individually immersed into 200 ml of either water or PBS in 500 ml beakers. 3 ml solution from aforementioned release set-up (beaker) was collected after periodic timescales including t=0 min, and the UV-Vis spectroscopic data was measured at λ_{max} of 276 nm. After measurements, the solutions were poured back into the original reservoirs to maintain accumulative release conditions. Studied drug concentrations were well within the sink conditions[51].

Entrapment efficiency (%) and drug loading (%) in cubosomes and bulk cubic phase

1 gm of drug (2%) loaded dispersed cubosomes were added to 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 further 10 min. Majority of the cubosome particles were removed by this method; however, the emulsion retained the opacity. The remaining lipid was separated by using Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-3 membranes (NMWL 3 KDa, Millipore, USA) with 20 min centrifugation at 13,200 rpm. The separated aqueous phase was diluted with water and the absorbance at 276 nm was measured using UV-Vis spectroscopic technique. The entrapment efficiency (EE) and drug loading (DL) was determined using following formulae[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 non-entrapped 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 for other cubosome dispersions (with 4, 6, and 10% drug) were determined in the same manner as descried above. The excess water (aqueous phase) from drug-loaded bulk cubic phase system was separated simply by decanting into empty cuvettes followed by recording their 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 above formulae (*eqs. 1* and *2*).

Particle size analysis of cubosome dispersions

The mean particle size and size distribution of cubosome dispersions were measured using 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 bulk and dispersed lipid systems (Figure 2). The SAXSpace instrument (Anton Paar, Graz, Austria) at University of Leeds was employed for this purpose. The details of the instrument and measurement are published earlier[53]. The instrument is based on Cu-anode operating at 40 kV and 50 mA. Samples were measured using capillary sample holder controlled at 25±0.1°C. Typical exposure times of 300 sec 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. The bulk DU formed *Pn3m* type bicontinuous cubic phase in excess water[49], which was retained upon addition of 10% drug (Figure 2a). In case of dispersed lipid systems, the addition of stabilizer molecules usually cause phase transition into *Im3m* type bicontinuous cubic phase[20, 54-55] (Figure 2b).

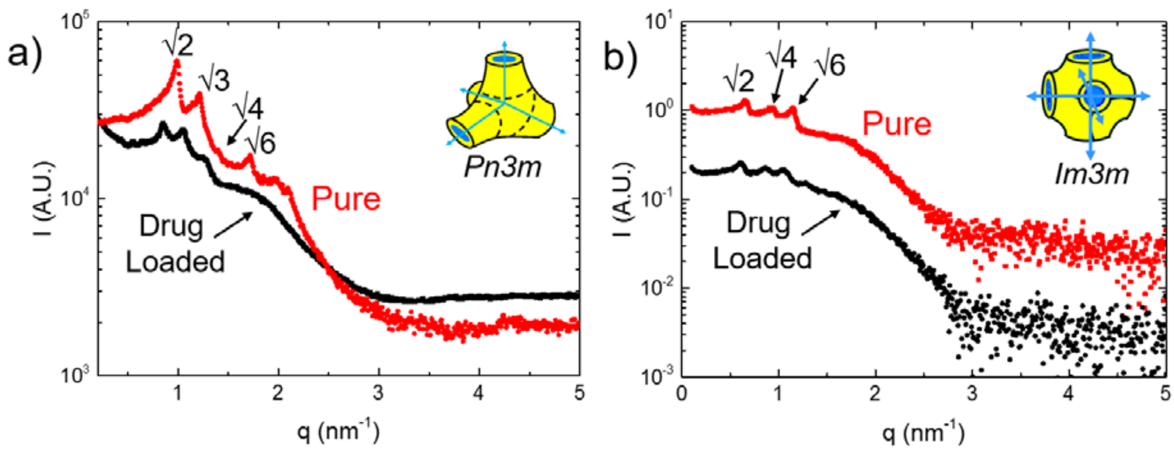

Figure 2: Detection of the type of lipid self-assembly using SAXS: a) *Pn3m* cubic phase was observed in pure and drug-loaded (10% drug) bulk lipid system. b) *Im3m* cubic phase was observed in pure and drug-loaded (10% drug) dispersed cubosome system. Characteristic Bragg's diffractions for *Pn3m* (√2, √3, √4, √6) cubic phase and *Im3m* (√2, √4, √6) cubic phase are shown near corresponding peaks. Aqueous channels are indicated by arrows.

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

Drug in Dispersion (Wt% per Wt of lipid)	**Average Particle Size nm**	**PDI (Poly dispersity 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

Table 1. Particle size and polydispersity indices of native and drug-loaded cubosomes measured by dynamic light scattering technique Zetasizer Nano ZS.

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

Drug loading	**EE bulk cubic phase in excess water**	**EE cubosome dispersion**	**DL cubic phase**	**DL cubosomes**
Wt %/Wt of lipid	**%**	**%**	**%**	**%**
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

Table 2. Entrapment efficiency (EE) and Drug Loading (DL) in bulk lipid phase and dispersed cubosomes.

Drug release from bulk cubic phase

Referring to the standard calibration curves obtained by dissolving various concentrations of a 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 in water (Figure 3) and in PBS (Figure 4) for bulk cubic phase.

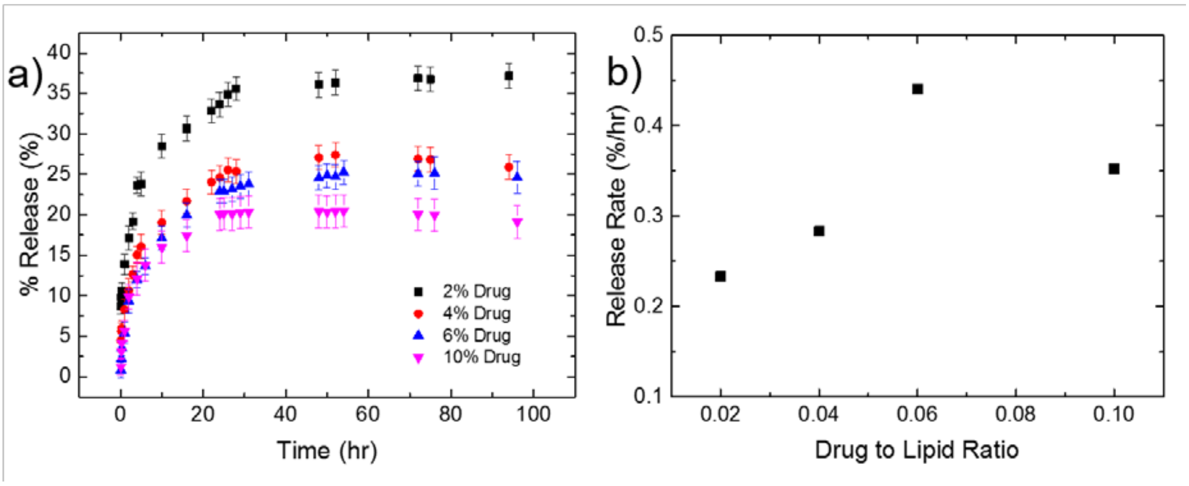

Figure 3: Drug release from bulk cubic phase in water: a) Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) are shown against time in hours for 2, 4, 6 and 10% drug. b) Curves in a) were fitted with Korsmeyer-Peppas equation to obtain the release rates (%/hr),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

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

$$M_t / M_\infty = K t^n \qquad (3)$$

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 drug release in water medium became insignificant after about 30 hrs (Figure 3a) but it was gradual and well-detectable up to about studied 96 hrs in case of PBS release medium

(Figure 4a). Slopes of the fit lines, i.e. release rates in %/hr follows a linear trend except for 10% drug sample (Figures 3b and 4b). The corresponding slopes of these lines were comparable: 0.05198 %Wt%hr^{-1} for release in water (Figure 3b) and 0.04701 %Wt%hr^{-1} for release in PBS medium (Figure 4b). Both release rates are dependent on the drug concentration and follow first-order kinetics.

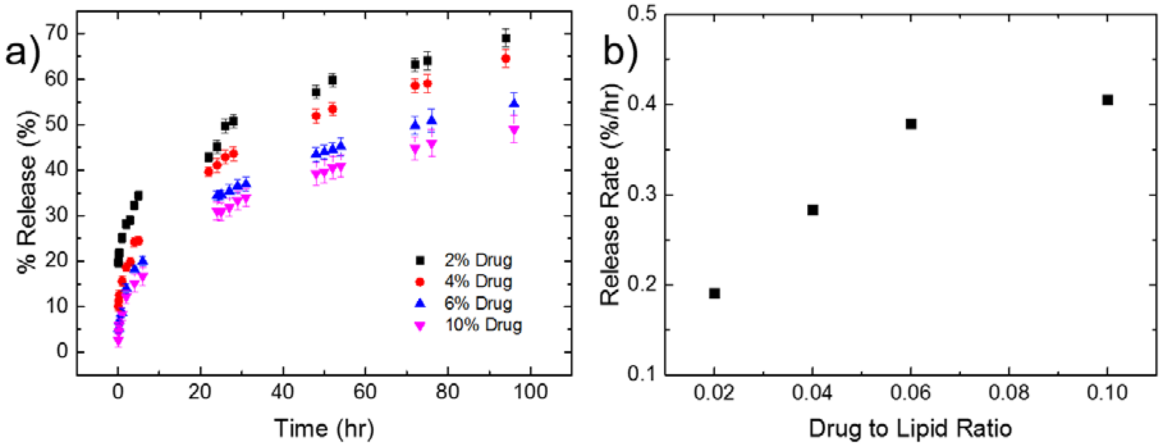

Figure 4: Drug release from bulk cubic phase in PBS: a) Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) are shown against time in hours for 2, 4, 6 and 10% drug. b) Curves in a) were fitted with Korsmeyer-Peppas equation to obtain the release rates (%/hr),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

Drug Release from dispersed cubosomes

The release trends from cubosomes (Figure 5) virtually followed similar release patterns as from bulk cubic phase (Figures 3 and 4). The release in water saturated after about 30 hrs whereas it continued gradually in case of PBS.

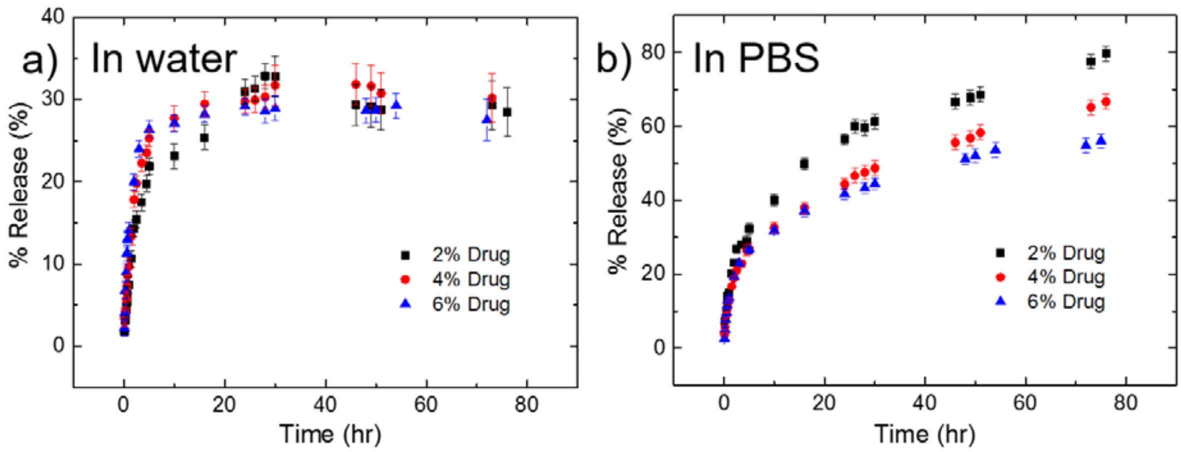

Figure 5: Drug release from dispersed cubosomes in a) water and b) PBS: Percent (%) drug release calculated from corresponding absorbance values (at λ_{max} of 276 nm) is plotted against time in hours for 2, 4, 6 and 10% drug/lipid for release in water a) and in PBS b).

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 log-log scale, both in water and in PBS, were fitted using Korsmeyer-Peppas equation (*eq. 3*)[57] with typical R^2 values >0.93.

Sustained drug release from 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 was faster than the release from bulk cubic phases. The release of 2% drug from bulk cubic phase was found to be three-folds (0.233 %/hr) sustained than the release from cubosomes (0.658 %/hr) when measured in water; whereas it was twice more sustained from bulk cubic phase (0.190 %/hr) than from cubosomes (0.402 %/hr) in PBS. The same trend was also observed for the release rate values of 4 % drug in water (0.283 %/hr for bulk cubic phase and 0.637 %/hr for cubosomes) and in PBS (0.282 %/hr for bulk cubic phase and 0.407 %/hr for cubosomes). The releases for 6% and 10% drug from cubosomes were also faster than the releases from bulk cubic phases, both in water and PBS release media.

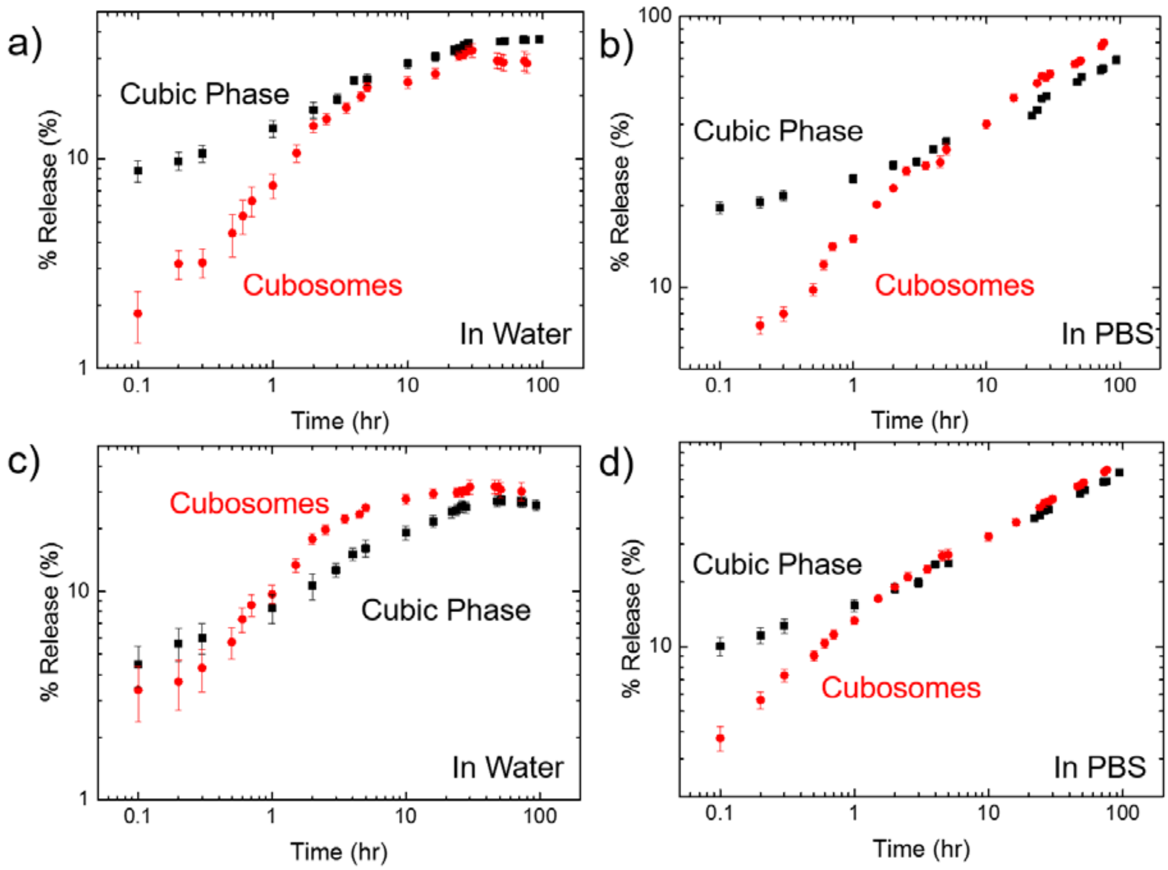

Figure 6: Comparison of drug release from bulk cubic phase and cubosomes: % release for 2% drug in a) water and b) PBS and 4% drug in c) water and d) PBS are shown with time in hrs.

Fittingness of Korsmeyer-Peppas equation to almost all curves in both lipid systems helps to understand the underlying release mechanisms. Analysis of the exponent *n* (*eq. 3*) suggest that the release mechanism is a Fickian diffusion when *n* value is less than or equal to 0.45 ($n \leq 0.45$). The release from bulk cubic phase, both in water and in PBS followed this mechanism. Apparent Fickian diffusion is known to contribute in sustaining the 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 the lengthy aqueous network assimilated by long and continuous lipid bilayer architecture. On the contrary, the release from cubosomes is rapid and less sustained (Figure 6). This is due to smaller (< 200 nm, ***Table 1***)[53] particle size correlated to smaller length scales for the drug transport, and too high surface area accountable to the burst release kinetics[59]. The release from cubosomes in water displayed *n* value below 0.45 but for the release in PBS the *n* value was greater than 0.45 (*n*=0.63) depicting anomalous or non-Fickian diffusion kinetics[57-58].

A few recent reports demonstrate that the release rates from lipid systems can be controlled by fine-tuning nanostructural type and dimensions of the self-assembled phases[30-32, 60-62]. For instance, if the nanostructure is hexagonal phase, the release can be more controlled in comparison to the open structured cubic nanostructures[34, 63]. Moreover, the release could be administered via stimuli responsive (pH, light or temperature induced) triggers[63-65]. We have recently demonstrated that it is possible 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 cubosomes, burst release and finally the dissolution of the hydrogel matrix[56].

The nanoscale design and dimensions of the cubic self-assemblies further influence the release kinetics as follows. The lipid used in this work forms *Pn3m* type cubic phase in pure water[49] (Figure 2a). This phase exhibits four aqueous channels (meeting at tetrahedral angle) whereas the internal self-assembly of cubosomes is found to be *Im3m* phase (Figure 2b) consisting six aqueous channels (meeting at right angles). Moreover, the aqueous channels of *Im3m* phase are usually larger than *Pn3m* phase for the same lipid system, see ***Table 3***. Largely hydrophilic block co-polymer stabilizers, similar to F127 used in this work, are known to alter the average molecular shapes of cubic phase forming lipids[20, 54]; most common result is the formation of *Im3m* phase in the internal cores of dispersed cubosomes[55].

Physical form of lipid system	**Concentration of drug Wt% per 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	*Pn3m*	4	89.5	37.9
Bulk cubic phase	10	*Pn3m*	4	103.2	48.7
Dispersed cubosomes	0	*Im3m*	6	135.6	50.9
Dispersed cubosomes	10	*Im3m*	6	146.7	57.8

Table 3. Nanoscale dimensions of lipid cubic phases formed in bulk system and dispersed systems, before and after drug loading. Lattice parameters and diameters of aqueous channels were calculated according to the formulae listed in reference[67].

Encapsulation of a drug further elevates diameters of aqueous channels by about 22% in bulk cubic phase while 12% in cubosomes (***Table 3***). Thus, the number of aqueous channels and their sizes are greater in the cubic phase (*Im3m*) of cubosomes as compared to the bulk cubic phase (*Pn3m*), which theoretically contribute to rather rapid release from cubosomes.

Efficient release in PBS than in water

The drug release from all samples, in bulk cubic phases and cubosomes, in PBS was far more efficient (more than double in some cases) than the release in water (Figure 7, ***Table 4***). PBS medium (pH ~7.4) is generally referred to be the physiological condition where drug release kinetics is usually different than in ambient and pure conditions.

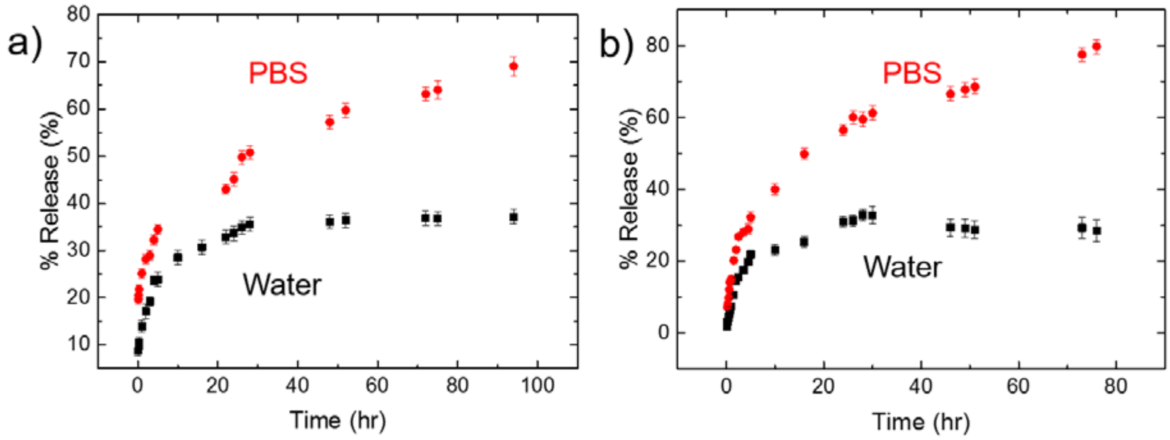

Figure 7: Drug release in PBS compared to water: a) Percent (%) drug release in water (solid squares) and in PBS (solid circles) for 2% drug/lipid is shown for a) bulk cubic phase and b) cubosome system. Note that the representative data plots for 2% drug/lipid samples are shown here; for remaining data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

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

Table 4. The % release at 24 hrs from both lipid systems in water and in PBS.

Main advantages of employing PBS for release studies include the retention of constant pH, prevention from denaturation and/or conformational changes, resembling the bodily conditions (isotonic) and nontoxicity to cells[68]. However, biological buffers, and also associated pH affects the membrane properties[68-69]. It was envisaged that the buffer molecules interact with hydrophilic lipid head groups and intercalate within the head group region facilitating the reduction of membrane bending modulus and increase in 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 with the continued and efficient release from lipid carriers in PBS medium in contrast to the low and ceased release in pure water medium (Figure 7, ***Table 4***).

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

Conclusions and perspectives

In this work we have compared the release properties of two different lipid nanocarrier systems. Bulk lipid cubic phase and cubosomes were studied under similar conditions of drug to lipid ratios and the volumes of release media. The release in both lipid systems followed a mechanism described by Korsmeyer-Peppas equation revealing the Fickian diffusion through the porous matrix[57-58]. However, the diffusion from cubosomes in PBS medium 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 from the efficient and rapid release in PBS medium compared to the almost ceased release in water medium (Figure 7).

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

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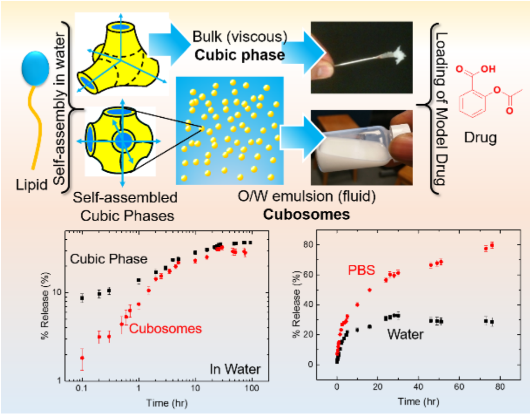

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