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Journal of Colloid and Interface Science 260 (2003) 404–413

JOURNAL OF Colloid and Interface Science

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Enhanced loading of water-soluble actives into bicontinuous cubic phase liquid crystals using cationic surfactants

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Received 3 June 2002; accepted 18 September 2002

Abstract

Over the past few years, bicontinuous cubic phase liquid crystals have been investigated for their applicability to controlled delivery of active ingredients. These liquid crystals have a unique structure of interpenetrating channels of water and lipid that provides compatibility with water-soluble, lipid-soluble, and amphiphilic active ingredients. Actives tend to be stable in the matrix and the structure provides control over their release. However, loading of water-soluble actives is difficult. It is especially problematic for cubic phase liquid crystal dispersions (cubosomes) given the large fraction of bulk water present. The inherent problem reflects the preference of the water-soluble actives to associate with water rather than with the liquid crystals. Ideally, the properties of the liquid crystal can be tailored to enhance the association of the liquid crystal with the active, thereby increasing loading. It is found that the inclusion of surfactant into the liquid crystal can provide this function. This work illustrates the enhanced loading of negatively charged, water-soluble active ketoprofen by the inclusion of positively charged surfactants into the liquid crystal. Loading differences resulting from the inclusion of dioctadecyl dimethyl ammonium chloride (DODMAC) and dioctadecyl ammonium chloride (DOAC) into the liquid crystal demonstrate that the magnitude of the enhancement is dependent on the surfactant concentration and the steric nature of its head group. The upper limit of the enhancement is explored by the inclusion of di(canola ethyl ester) dimethyl ammonium chloride (DEEDAC) formulated to greater than 20 wt% and demonstrates an order-of-magnitude enhancement over previous reports. This work provides a practical demonstration of functionalizing cubic phase liquid crystals and lays the framework for future work.

Keywords: Cubic phase liquid crystals; Drug delivery; Surfactants; Bicontinuous

1. Introduction

Surfactant and polymer systems form supra-assemblies, which are extensively exploited as active delivery vehicles [1]. These systems include liquid crystalline aggregates (e.g., liposomes and cubosomes) or cross-linked gel networks (hydrogels) that load, stabilize, and eventually deliver active ingredients. The potential for utilizing a particular active with a vehicle depends on the physicochemical properties of both. To achieve therapeutic effects, it must be possible to load sufficient amounts of the active, which largely depends on the interaction of the vehicle and active. Further, the integrity of the active must be retained through all stages: preparation, storage, and use. The release rate of actives must be controlled to achieve optimal drug release profiles, while ease of preparation and vehicle stability must also be considered. An optimal delivery vehicle must successfully encompass all these properties.

Liposomes (or vesicles) exhibit a number of properties that make them useful as vehicles for drug delivery; however, structure and stability limit their broad applicability. The use of vesicles dates back several decades and includes such diverse applications as local anesthetics [2], cancer therapy [3], gene therapy [4], and facilitating the transport of actives through the skin [5]. The structure of vesicles conjures images of a balloon-like thin membrane (a.k.a. lipid bilayer) enclosing a large amount of solution. Liposomes are generally fabricated by subjecting an aqueous dispersion

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0021-9797/03/$ – see front matter
doi:10.1016/S0021-9797(02)00016-4

of insoluble lipids to high-shear treatment, ultrasonication, or high-pressure homogenization [6]. The location of the actives in vesicles is dictated by the physicochemical properties of the active. Hydrophilic actives locate in the enclosed volume, where release is governed either by rupture of the vesicle (the most elegant example is the release of neurotransmitter in the synaptic cleft [7]) or by slow diffusion of the active through the bilayer skin of the vesicle. Hydrophobic actives locate in the lipid bilayers where it is possible to accommodate only several mole percent in the bilayer. Given the very small volume fraction of bilayer in vesicle dispersions, this can be a formidable limitation. Additionally, most vesicles are not at thermodynamic equilibrium [8] and are subject to change over time.

Hydrogels also exhibit a number of properties that make them useful for drug delivery [9]; likewise, however, their broad applicability for uptake and release (e.g., dermal patches and drug delivery) is generally limited by structure. They are formed in aqueous solutions from cross-linked insoluble polymers including polyesters, polyacrylic acid, polylactic acid, and polyacrylamide [10]. The structure of hydrogels conjures images of an interconnected web immersed in solution. Consequently, hydrogels can only entrap water-soluble actives in the interstitial fluid. Even with water-soluble actives, loading can be problematic. Hydrogels are generally soaked in a solution of the active that slowly diffuses into the matrix, the rate of which is governed by the size of the actives relative to the swollen matrix. Gels can be chemically cross-linked in the presence of an active. This is problematic, however, as the conditions necessary for polymerization often lead to denaturation or destruction of the active [11,12]. The addition of charge to the gels enhances the loading of actives by enhancing the ionic interactions between the active (e.g., protein) and the ionized polymer network [13]. The release of actives is a function of polymer concentration and type, drug interaction with the gel structure, and the presence of additives. Release from hydrogels can be difficult to predict since their random pore size distribution is difficult to control.

Bicontinuous cubic phase liquid crystals have many properties that make them appealing as a more universal vehicle for drug delivery than liposomes and hydrogels. Luzzati et al. [14] first documented the bicontinuous liquid crystal phase with the geometric model supplied later by Scriven [15]. It is only in the past decade that they have been examined for drug delivery [16]. A short list of applications includes the delivery of actives for periodontal disease [17] and implants [18] via in vivo [19] and topical delivery [20], and as bioadhesives [21]. A recent review by Drummond and Fong [22] provides an extensive listing of drug-related applications. The promise for these liquid crystals is found in their unique bicontinuous liquid crystal structure. The surfactant assembles into bilayers that are twisted into a periodic, three-dimension periodic minimal surface [23] forming a tightly packed structure that is “honeycombed” with bicontinuous domains of water and lipid. Consequently, the structure can simultaneously accommodate water-soluble, lipid-soluble, and amphiphilic molecules. A number of cubic phase liquid crystals have been proposed by Luzzati et al. [24]; however, three bicontinuous liquid crystal structures, P_{n3m} (D-surface), I_{a3d} (G-surface), and I_{m3m} (P-surface), are common (Fig. 1) and can be described in terms of nodal surfaces [25]. Bicontinuous cubic phases are found in natural lipids [26], cationic [27] and nonionic surfactants [28], and polymer [29] systems, although the lipid most widely used to construct bicontinuous cubic phases is the monoglyceride monoolein. Following the phase diagram published by Qiu et al. [30], monoglycerides spontaneously form bicontinuous cubic phases upon the addition of water, are relatively insoluble (allowing the formation of colloidal dispersions of cubosomes), and are resistant to changes in temperature. Actives can be loaded by direct addition to melted lipid or water (i.e., before hydration) or by diffusion into the structure after it is formed. The structure generally maintains the efficacy of the actives and can actually help stabilize actives such as vitamins [31] and proteins [32]. There is a practical upper limit to loading lipid-soluble actives; typically about 10 wt% (relative to the cubic phase), governed by the loss of cubic phase structure upon addition. The bicontinuous liquid crystal structures are thermodynamically stable, lasting indefinitely, provided there is no hydrolysis of the lipid [33]. Colloidal dispersions of the liquid crystal (cubosomes) can be stabilized by the addition of polymers [34]. They also possess the potential for controlled delivery of actives, where diffusion is governed by the tortuous diffusion of the active through the “regular” channel structure of the cubic phase [35]. Bicontinuous liquid crystals hold tremendous potential for the controlled delivery of actives and have inherent advantages over alternative vehicles.

The objective of this research is to broaden the utility of bicontinuous cubic phase liquid crystals through functionalization, as demonstrated here by increasing the loading of water-soluble actives. Most previous reports attempt to utilize the inherent properties of the liquid crystal cubic phase; their properties are “as they are” with little ability to change. The approach here tailors the properties by including surfactants into the cubic phase liquid crystal. Surfactants are chosen so that the tail of the surfactant is miscible in the bilayers and the charged head group resides in the water channels, effectively functionalizing the liquid crystal by enhancing the interaction between the active and matrix. Lindell et al. [36] examined the effect of a charged phospholipid functionalizing group, distearoylphosphatidylglycerol, on the release behavior of timolol maleate from cubic phase at molar ratios of 2:1 and 4:1 additive:active and observed increases in equilibrium loading of 20 and 30 wt%, respectively. However, the levels of surfactant and active are unrealistically small for many practical applications, such as cosmetics. Further, the kinetics of release were relatively unaffected by functionalization. Because the cubic phase is limited in its ability to accommodate additives, it is necessary to opti-

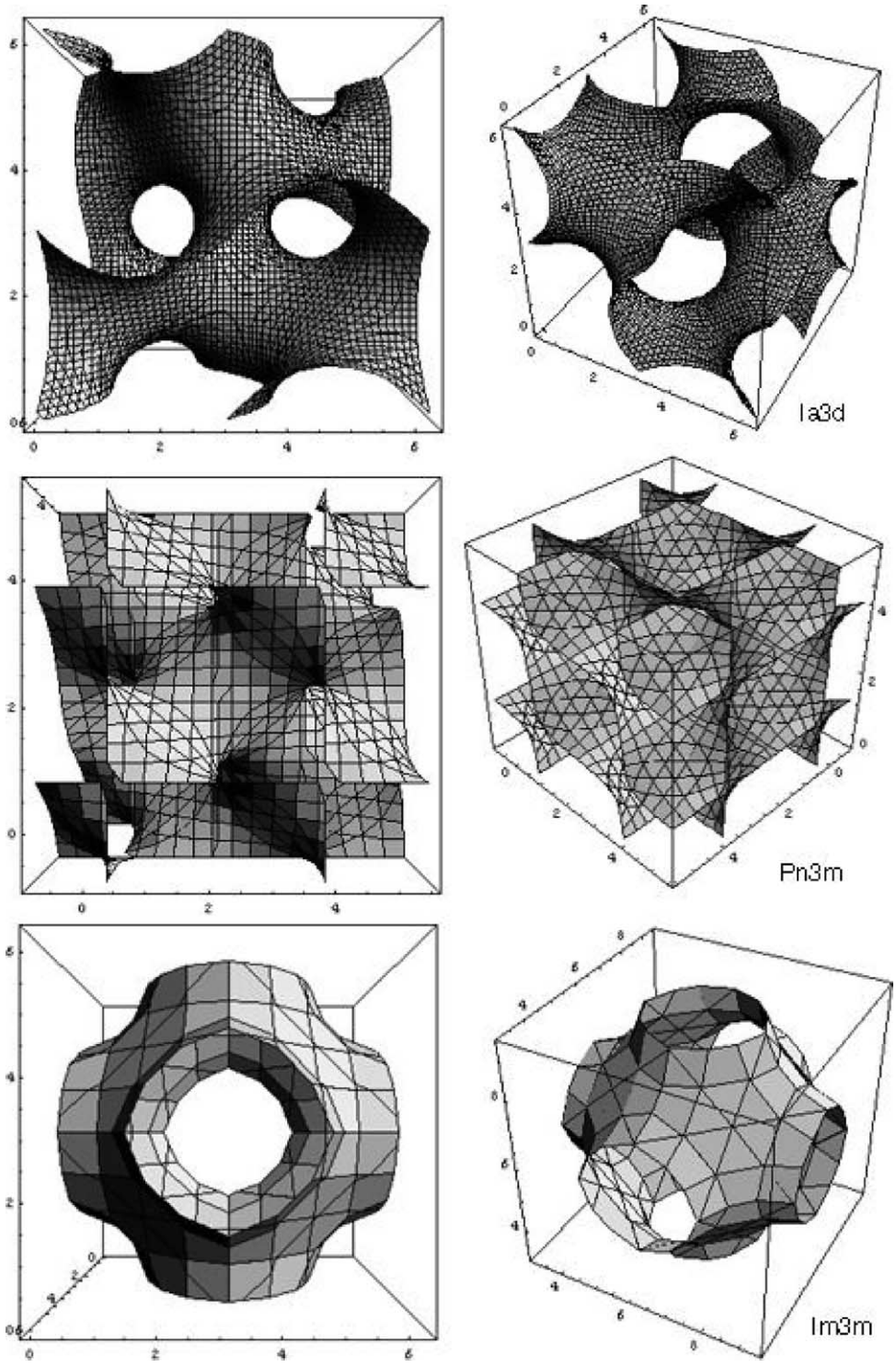


Fig. 1. Renditions of the structures of the different cubic phase liquid crystals. The G-surface (top) belongs to the I_{a3d} space group (No. 214) and is approximated by the expression $\sin x \cos y + \sin y \cos z + \cos x \sin z = 0$. The D-surface (middle) belongs to the P_{n3m} space group (No. 227) and is approximated by the expression $\cos(x-y)\cos z + \sin(x+y)\sin z = 0$. The P-surface (bottom) belongs to the I_{m3m} space group (No. 221) and is approximated by the expression $\cos x + \cos y + \cos z = 0$. Please note that the space group assignments refer to the surfaces generated from the nodal functions [25] and are slightly different from the space groups assigned by Luzzati et al. [24] for the actual bicontinuous structures, which are No. 230, No. 224, and No. 229, respectively.

mize the efficiency of the functionalizing molecule and its level of loading as well. By examining the partition (and release) from various combinations of cationic surfactants with ketoprofen, this work addresses both of these concerns and examines the upper limits of the functionalization process.

2. Materials and methods

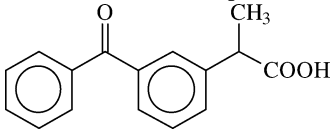
2.1. Cationic surfactant

Both DODMAC and DOAC were synthesized at Procter & Gamble and determined to be greater than 99% pure. The DEEDAC (Goldschmidt) is a commercial-grade surfactant containing about 85% active with the remainder consisting of amines (with fewer than two methyl groups), fatty acids, unreacted amines, and a small amount of antioxidants and metal chelant preservatives. The lipid chains are made from a touch-hardened canola feedstock and are highly unsaturated, having an iodine value (IV) of about 90.

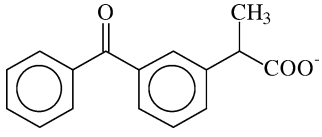
2.2. Ketoprofen

Ketoprofen, an analgesic used to reduce inflammation of the joints and muscle tissue, was purchased from Wyckoff Inc. (Catalytica Pharmaceuticals). Ketoprofen is a weak acid—it is protonated at low pH values, making it very hydrophobic and insoluble in aqueous buffers, and it ionizes at high pH values, making it somewhat soluble in aqueous buffers. The solubility of the neutral form in water is 0.0142 wt% [37]; the solubility of the ionized form in pH 10 buffer is higher.

Acid form of ketoprofen



Base form of ketoprofen



2.3. Dissociation constant for ketoprofen

The dissociation constant for ketoprofen is difficult to obtain since the material is relatively insoluble in water; consequently, the dissociation constant was determined in ethanol–water mixtures and these data are regressed to a solution with no ethanol. Stock solutions were prepared at 40, 50, 60, 70, and 80 wt% ethanol in water. A fraction of these solutions was added to NaOH pellets (Sigma Cat #S-0899) creating approximately 0.1 molal solutions of a known ethanol–water concentration. These solutions were standardized against KHP (Sigma #P-3792) using a phenolphthalein end point. A second set of solutions was prepared by dissolving approximately 0.7 g of ketoprofen in each ethanol–water mixture. These solutions were titrated with the standardized NaOH solution. The weak-acid titration curve was assembled using a Corning 430 pH meter to determine the pH with each caustic addition. The dissociation constant for each ethanol–water mixture was taken from the pH value at the midpoint of the titration curve. The best-fit line to the data demonstrates a change in pKa of 0.0228 $(wt\%)^{-1}$ per change in ethanol–water composition (Fig. 2). The effective pKa for aqueous solutions was determined from the regression of the data to 0% ethanol and found to be $pKa = 4.70$. This value is reasonable given the values between 3.7–4.45 reported in the literature [38] and close to those of similar soluble acids: propanoic acid, $pKa = 4.88$, acetic acid, $pKa = 4.75$, and benzoic acid, $pKa = 4.20$.

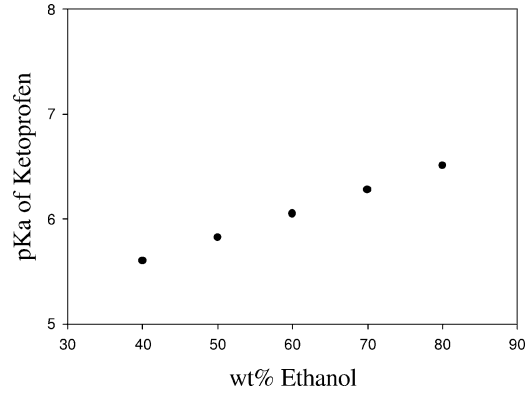


Fig. 2. The dissociation constants for ketoprofen measured in various ethanol–water mixtures. The dissociation constant in aqueous solutions is determined by regression to 0% ethanol.

2.4. Preparation of buffers

Buffers were prepared by mixing solutions of the following compounds:

pH 4 buffer: 0.2 M acetic acid (J.T. Baker Cat #9508-01) and 1 N sodium hydroxide (J.T. Baker Cat #5635-02),
pH 5 buffer: 0.2 M acetic acid and 1 N sodium hydroxide,
pH 6 buffer: 0.2 M acetic acid and 1 N sodium hydroxide,
pH 7 buffer: 0.2 M NaH_2PO_4 (Aldrich Cat #7558-80-7) and Na_2HPO_4 (J.T. Baker Cat #3824-01),
pH 8 buffer: 0.2 M NaH_2PO_4 and Na_2HPO_4,
pH 9 buffer: 0.2 M NaH_2PO_4 and Na_2HPO_4,
pH 10 buffer: 0.2 M $Na_3PO_4{\cdot}12H_2O$ (J.T. Baker Cat #3836-01) and Na_2HPO_4.

All buffers were diluted to 0.1 M.

2.5. UV-vis calibration of ketoprofen in octanol and buffer

Six samples between 0.000 and 0.005 wt% ketoprofen in octanol were prepared. The UV-vis absorption spectra were measured in a Perkin–Elmer Lambda 40 spectrometer. There is a well-resolved peak centered at ∼254 nm associated with the ketoprofen. The height of the peak is determined using GRAM 5.0 software after subtracting the baseline. A Beer's law plot was constructed with the absorption data and the best-fit slope of the plot was determined to

be 352.6 (wt%)$^{-1}$, which corresponds to the weight-based absorptivity constant, ε_b.

Four samples between 0.000 and 0.005 wt% ketoprofen in pH 10 buffer were prepared. A Beer's law plot was constructed with the absorption data and the best-fit slope of the plot was determined to be 373.1 (wt%)$^{-1}$, which corresponds to the weight-based absorptivity constant.

2.6. Partition experiments with octanol

In 40 ml bottles, 0.03 g of ketoprofen powder was dissolved in 10 g of octanol (Aldrich Cat. #36,056-2), creating a 0.003 wt% solution. Ten grams of buffer were added to the bottles. The solutions were shaken and allowed to separate and equilibrate for 12 h. From the top of the solution, 1 ml of octanol was removed and measured for ketoprofen with the UV-vis Perkin–Elmer Lambda 40 spectrometer. The concentrations were determined using the aforementioned calibration curves and GRAMS 5.0.

2.7. Partition experiments with cubic phase

The desired percentages of ketoprofen were prepared by dissolving the appropriate weights of the acid form of ketoprofen into liquid monoolein (when heated and cooled, the solution remains liquid for hours). Buffer was added to the top of the mixture to create final solutions, which were 50:50 weight fractions buffer to gel. The mixtures were allowed to equilibrate for 24 h. For more dilute solutions, the concentration was determined directly from the UV-vis of the extracted buffer. For more concentrated samples, extracted buffer was removed and systematically diluted to obtain absorbance readings below 1.0.

2.8. Small angle X-ray scattering (SAXS)

SAXS was performed on samples with Cu$K\alpha$ radiation ($\lambda = 0.154$ nm) generated with a Rigaku RU-300 rotating anode. The generator was operated at 40 kV and 40 mA with a 0.2×0.2 mm focal size (a 0.2×2 mm filament run in point mode). The patterns were collected with the Siemens 2-dimensional small angle scattering system, which consists of a HI-STAR wire detector and an Anton Parr HR-PHK collimation system. Collimation is achieved with a single 100-μm diameter pinhole 490 mm from the focal spot. The size of the focal spot restricts beam divergence. A 300-μm guard pinhole was placed 650 mm from the focal spot, just in front of the sample. The detector was placed a distance of 650 mm from the sample. Ni filters were used to eliminate the $K\beta$ radiation. Because of the small beam size and large sample-to-detector distance, two-dimensional profiles (q_x, q_y) could be obtained with a minimum of instrumental smearing, so no smearing corrections were employed.

2.9. Partition experiments with cubic phase functionalized with surfactant

To measure the partitioning with enhanced cubic phase liquid crystals, surfactant (0-, 10-, 15-, and 30-mole excess of surfactant, relative to the active) and ketoprofen were added to the liquid monoolein and stirred until they dissolved. The surfactant concentrations were less than 1 wt%. Once completely mixed, buffer was added above the sample. The amount of buffer was again fixed to create final solutions that were 50:50 weight fractions buffer to gel. The vials were left for 24 h (found to be sufficient for equilibration) and UV-vis readings were taken on the liquid above the gel.

To ensure that the addition of the surfactants did not significantly alter the cubic phase liquid crystal structure, SAXS patterns were used to confirm the structure. For these stated ratios of the monoolein and water, the liquid crystal was determined to have P_{n3m} symmetry. This symmetry provides six strong reflections assigned to the following Miller indices: [110], [111], [200], [211], [220], and [221] [39]. For cubic phase symmetry, a plot of peak position versus $\sqrt{h^2 + k^2 + l^2}$ generates a straight line with a slope inversely proportional to the lattice parameter. The inclusion of DODMAC and DOAC does not change the structure of the cubic phase liquid crystal, as illustrated by the SAXS data for DOAC (Table 1). The SAXS data also suggest that at higher surfactant concentrations, a solubility limit is reached in the liquid crystal and the surfactant begins to crystallize. The inclusion of the DEEDAC and ketoprofen at higher concentrations forces a change in the structure of the cubic phase liquid crystal from P_{n3m} to I_{a3d} symmetry. This is evident from six strong reflections which are assigned to the following Miller indices: [211], [220], [321], [400], [420], and [332] (Table 2). Such structural changes are common. It is uncommon, however, that the surfactant can be added to such a large degree without change to a noncubic phase structure. It is also curious that the lattice parameter does not appreciably change at lower concentrations, but increases at

Table 1
SAXS data for the inclusion of DOAC surfactant in cubic phase monoolein

Sample	Slope (Å^{-1})	R-value (of fit)	Lattice parameter (Å)
0.16% DOAC·HCl	0.0644	0.99999	97.6
0.32% DOAC·HCl	0.0636	0.99996	98.8
0.95% DOAC·HCl	0.0639	0.99999	98.3
1.22% DOAC·HCl	0.0629	1.00000	99.9
3.92% DOAC·HCl	0.0629	0.99994	99.9

Table 2
SAXS data for the inclusion of DEEDAC and ketoprofen in cubic phase monoolein

Sample	Slope (Å^{-1})	R-value (of fit)	Lattice parameter (Å)
5% DEEDAC + 2% ketoprofen	0.0406	0.99991	154.5
10% DEEDAC + 2% ketoprofen	0.0412	0.9998	152.1
20% DEEDAC + 2% ketoprofen	0.0338	0.99792	185.5

higher concentrations. This is possibly due to charge–charge repulsions that might be anticipated once the surfactant concentration reaches a critical concentration in the bilayers.

2.10. Preparation of samples for controlled release

About 0.5 g of monoolein were melted into the bottom of a 100-ml glass cylinder. Surfactant and ketoprofen were dissolved into the melt. Sufficient buffer was added to the monoolein to form the cubic phase liquid crystal. Once formed, an additional 50 ml of buffer was added to the cylinder. An overhead propeller briskly stirred the solution above the liquid crystal. This arrangement was designed to have a 1-dimensional diffusion profile with the diffusion of ketoprofen through the gel as the rate-limiting step. Small aliquots of buffer were periodically removed, and the concentration of ketoprofen was determined in these solutions by UV-vis analysis.

3. Results and discussion

3.1. Partition of ketoprofen into octanol

The partition of ketoprofen into octanol is dependent on the pH, reflecting the ionization state of ketoprofen but does not simply reflect the trends predicted by the measured dissociation constant. Partition data were collected from pH 4 to pH 10 (Fig. 3). As anticipated, at low pH values essentially all the ketoprofen was in the octanol. Conversely, as the pH increased, most of the ketoprofen was found in the buffer above the octanol. Curiously, the shape of the partition curve does not track what might be anticipated solely on the basis of acid–base equilibrium. Assuming the pKa reflects the pH at which there are equimolar amounts of the acid and base, the 50% partition value would be expected when the pH = pKa. Instead this point is shifted to higher pH values. Implicit in this curiosity is that all ionized ketoprofen is only soluble in water and all neutral ketoprofen is soluble only in octanol. This is apparently incorrect, and the shift likely reflects the relatively high lipid solubility of the ionized species.

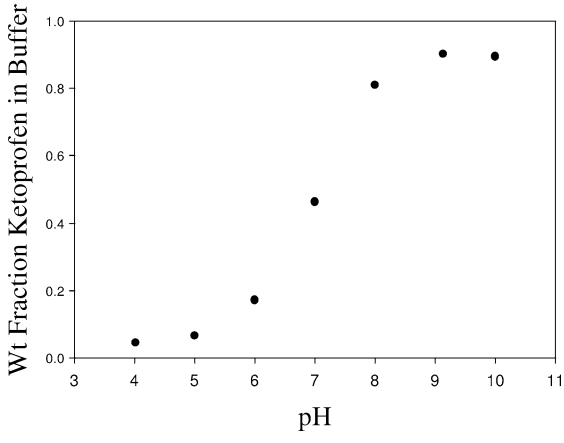


Fig. 3. The partition of ketoprofen into the buffer layer of a mixture of equal weights of buffer and octanol. The total amount of ketoprofen is measured to allow a maximum of 0.003 wt% in the buffer.

3.2. Expressing the partition coefficient with cubic phase liquid crystals

Ideally, the partition coefficient is expressed as a thermodynamically relevant quantity. The basic properties of thermodynamics (as relevant to partitioning) rely on the concept of phase equilibrium, requiring that the chemical potential of each component in adjacent phases be equal [40]. In ideal solutions, the chemical potential is directly related to the mole fraction of each component. Therefore, the partition coefficient in this work is expressed by the mole ratio of the active in the cubic phase liquid crystal and in the excess buffer above the gel. It is tempting to express partitioning in more elaborate terms by replacing the phase with the structure of the phase and defining locations of the actives within the phase structure. For example, actives within cubic phase liquid crystals are often discussed with respect to their position in the lipid bilayer or in the water channels [41]. While these are interesting questions, they cannot be addressed directly in the context of the partition coefficient. To derive a partition coefficient, a condition of equilibrium is stated,

$$\mu_c = \mu_b, \tag{1}$$

where the subscripts c and b reflect the cubic phase liquid crystal and buffer respectively. Then we define χ_b as the mole fraction in the buffer and $(1-\chi_b)$ as the mole fraction in the cubic phase. Expanding each expression for the chemical potential results in

$$\mu_b = \mu_b^0 + RT\ln(\chi_b), \tag{2}$$

$$\mu_c = \mu_c^0 + RT\ln(1-\chi_b), \tag{3}$$

where μ^0 is the chemical potential of the standard state. Solving for the mole fraction χ_b in each equation and taking the quotient, the expression reduces to

$$P = \frac{\chi_c}{\chi_b} = \frac{1-\chi}{1} = \mathrm{ext}\left(\frac{\Delta\mu^0}{RT}\right), \tag{4}$$

where $\Delta\mu^0$ reflects the chemical potential in the standard states or $\Delta\mu^0 = \mu_c^0 - \mu_b^0$. The partition coefficient is simply a statement of the difference in chemical potential of the ketoprofen in each phase. Accordingly, changes in partitioning can be explained and predicted on the basis of the changes in chemical potential associated with additional materials, such as surfactants. For example, it is known that oppositely charged surfactant interact to decrease the chemical potential of each, and would thereby increase loading. The partition coefficient can then be extracted from the experimental data by using a series of measurements and mass balance expressions. The partition coefficient can be

expressed as

$$P = \frac{\chi_c}{\chi_b} = \frac{M_c}{M_b}, \tag{5}$$

where M_b is the moles of ketoprofen in the buffer and M_c is the moles of ketoprofen in the cubic phase. By taking the experimental variables—molecular weight of ketoprofen, MW, weight of ketoprofen, W_k, weight of buffer, W_b, weight of monoolein, W_{mo}, and absorption of the ketoprofen in the buffer after equilibration, A_k—the moles of each component can be computed by first recognizing that added buffer will create the cubic phase at a ratio of 60 wt% monoolein and 40 wt% buffer with the excess in equilibrium, which also provides equal weights of each phase. Then the concentration of ketoprofen in the buffer can be determined from the Beer's law relationship by dividing the absorption by the weight-based absorptivity,

$$M_b = \frac{A_k}{37300} \frac{\left(W_b - \frac{2}{3}W_{\mathrm{mo}}\right)}{MW}. \tag{6}$$

Finally, the amount of ketoprofen in the cubic phase can be determined by mass balance,

$$M_c = (M_k - M_b) = \frac{(W_k - W_b)}{MW}. \tag{7}$$

The partition of ketoprofen between the cubic phase liquid crystal and buffer is similar to and different from the partition into octanol. The data plotted in Fig. 4 show the weight percentage of ketoprofen in buffer above the cubic phase. As expected, at pH 4, essentially all the ketoprofen is in the cubic phase. As the pH is increased, more ketoprofen is observed in the buffer. As with the octanol solvent, there is an apparent shift in pKa. In contrast, however, the data suggest that the ionized form is somewhat hydrophobic, as a much larger fraction is in the cubic phase liquid crystal than for comparable concentrations in the octanol. These data underscore the necessity of performing the partition experiments with the cubic phase liquid crystals, rather than octanol.

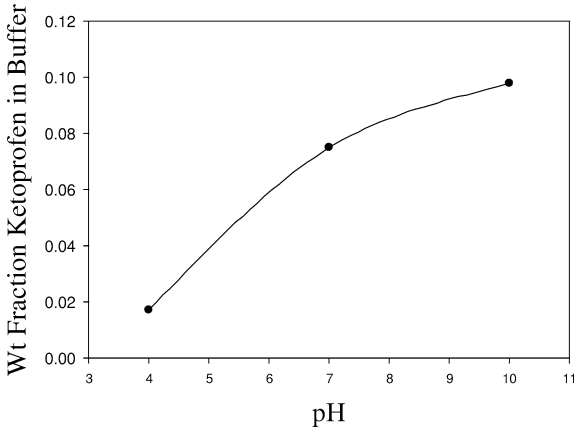


Fig. 4. The partition of ketoprofen into unmodified cubic phase gel for a system containing equal amounts of cubic phase gel and buffer. The total amount of ketoprofen is measured to allow a maximum of 0.003 wt% in the buffer.

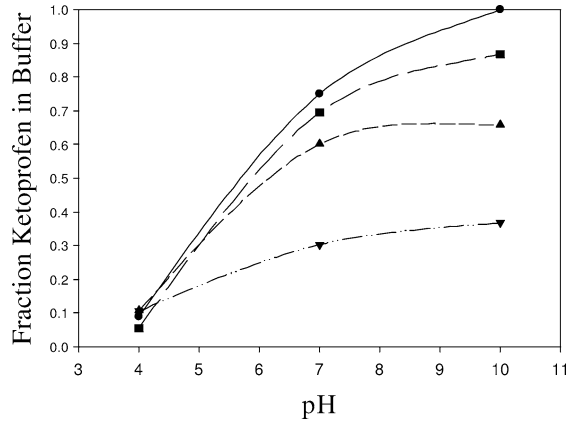


Fig. 5. The increase in ketoprofen concentration associated with the inclusion of DODMAC into the cubic phase gel. The decrease of ketoprofen in the buffer (and corresponding increase in the gel) is related to the amount of surfactant in the gel; the greater the concentration of DODMAC, the more ketoprofen resides in the cubic phase. The curves represent no addition (circles, solid line), 10 mole-fraction excess (squares, long dashes), 15 mole-fraction excess (triangles pointing up, short dashes), and 20 mole-fraction excess (triangles pointing down, dashes and dots).

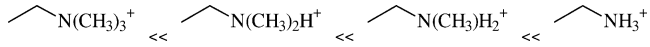
3.3. Effect of DODMAC on ketoprofen partitioning

The addition of DODMAC to the cubic phase increases the loading of ionized ketoprofen. Figure 5 illustrates the effect of surfactant addition on the partitioning of ketoprofen. At pH 4, there is little difference in partitioning regardless of the amount of added surfactant. The ketoprofen is unionized and is expected to be predominantly soluble in the lipid domains of the cubic phase. Conversely, at pH 7 and pH 10, ketoprofen is largely ionized and somewhat soluble. The amount of ketoprofen in the buffer decreases with increasing concentration of DODMAC. The effect on loading increases with an increase in DODMAC concentration presumably due to the increased number of positive charges in the gels. This is a substantial increase (up to 75%) in loading over non-surfactant-containing cubic phase.

3.4. Effect of DOAC on ketoprofen partitioning

DOAC is a weak acid, and for a reasonable comparison to DODMAC, it is necessary to measure the pH dependence of DOAC's ionization. This is achieved by fixing the pH in cubic phase liquid crystal samples that contain DOAC. They are titrated with specific amounts of NaOH: 100, 86.5, 72.8, 59.3, 45.4, 31.72, 18.3, 4.74, −9.0, −22.7, and −36.2 mol% of DOAC (negative in reference to excess titrant). The assumption is that the NaOH reacts stoichiometrically with the weak acid, which is typical. Several hours after the addition, the pH in the solution above the cubic phase is measured with a pH meter (Fig. 6). The pH in the solution above the cubic phase can be used to calibrate the ionization state of the DOAC, recalling that the chemical potential of the hydrogen ion in the solution (as measured by the pH meter) must equal the chemical potential of the hydrogen ion in the cubic phase at equilibrium.

DOAC does a significantly better job than DODMAC at increasing the loading of ionized ketoprofen. A similar partition experiment is preformed at pH 6, where essentially all the DOAC is protonated (positively charged) (Fig. 7). In contrast to the DODMAC, essentially all the water-soluble ketoprofen is absorbed into the cubic phase even at relatively low mole fraction excess. There is a relatively large increase in loading, relative to the DODMAC, which is likely indicatively of comparatively large interactions between the ketoprofen and surfactant. One way of accounting for the differences in interaction is to acknowledge that the head groups are significantly different in size between the two surfactants. DODMAC head groups are surrounded by methyl groups, which limit interactions with the ketoprofen. However, only protons surround DOAC, which allows for more substantial interactions (Fig. 8). The order of interactions might be anticipated as



The DODMAC and DOAC findings can now be applied to optimize the selection of functionalizing surfactant.

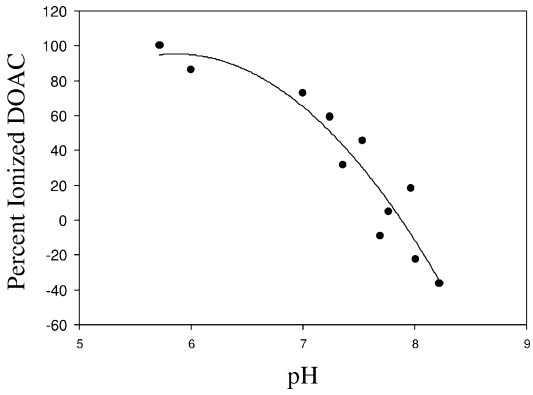


Fig. 6. The fraction of DOAC that is ionized within the cubic phase as a function of pH. The DOAC is titrated with known amounts of base into an equal weight mixture of water and cubic phase gel. The resulting pH was measured above the gel.

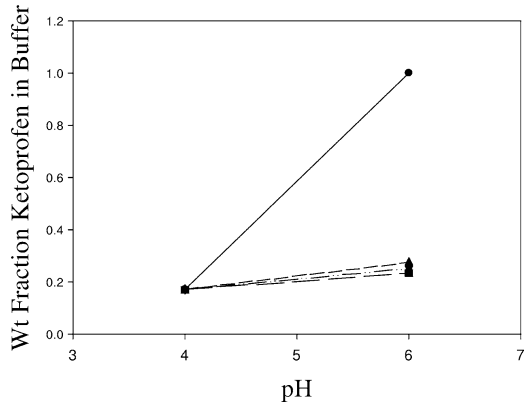


Fig. 7. The increase in ketoprofen concentration associated with the inclusion of DOAC into the cubic phase gel. The decrease of ketoprofen in the buffer (and corresponding increase in the gel) is related to the amount of surfactant in the gel; the greater the concentration of DOAC, the greater the ketoprofen in the cubic phase. The curves represent no addition (circles, solid line), 10 mole-fraction excess (squares, long dashes), 15 mole-fraction excess (triangles pointing up, short dashes), and 20 mole-fraction excess (triangles pointing down, dashes and dots). The experiments are only done below pH 6 to ensure all the DOAC is ionized. As evident when comparing to the DODMAC, the surfactant is far more efficient at increasing the uptake of ketoprofen.

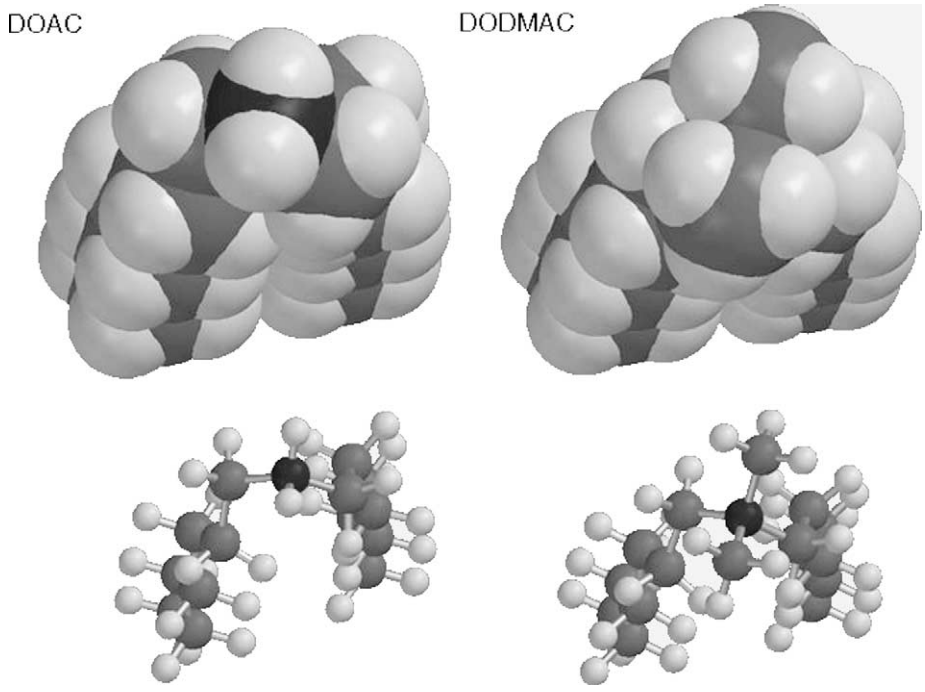


Fig. 8. Space-filling models of DODMAC (two figures on the right) and DOAC (two figures on the left). Clearly, the methyl groups obscure the nitrogen on DODMAC, lowering its enhancement of cubic phase loading. Conversely, the protons on the nitrogen on DOAC leave the charge relatively accessible.

3.5. Partitioning with DEEDAC

DEEDAC is ideal as a functionalizing surfactant since it has minimal effect on the cubic phase liquid crystal while, at the same time, it has maximum interaction with the active. The surfactant has a low Krafft temperature due to the high unsaturation level, which prevents surfactant crystallization [42]. Further, it has two long hydrophobic chains that decrease the water solubility of the surfactant, effectively anchoring it in to liquid crystal. In fact, it is so sparingly soluble that it does not form micelles in dilute solutions, instead exhibiting lamellar liquid crystal and monomer solution equilibrium. Additionally, its molecular shape (often described in terms of the critical packing parameter) lends to convenient insertion into bilayers resulting in very high loading levels (as high as 40 wt%). Finally, it is commercially available material, providing a practical and economic opportunity to functionalize the cubic phase liquid crystals.

The loading and release of ketoprofen is profoundly affected by the inclusion of DEEDAC. Controlled release curves for ketoprofen from a functionalized (containing 2 wt% ketoprofen and 20 wt% DEEDAC) and an unmodified cubic phase liquid crystal control are shown in Fig. 9. Both equilibrium partition and kinetic data can be extracted from these curves; each curve asymptotically approaches a constant weight-percentage at long times, which defines the equilibrium partition value, and the rate at which each curve approaches the partition value is a measure of the diffusion of active through the liquid crystal. From these data, it is obvious that both the partition value and diffusion are modified by the addition of the surfactant. To the first point, the inclusion of the surfactant alters the loading of ketoprofen from about 88 to about 97.5 wt%, which is a significant enhancement toward total loading of the liquid crystal. This is far more efficient than the enhancement illustrated by Lindell et al. [36], which stands at about 25% at the same surfactant-to-active mole ratio (about four to one). Despite the "crowded" head group associated with the DEEDAC, it is more efficient than distearoyl phosphatidylglycerol (DSPG) at enhancing the loading. To the second point, the inclusion of the surfactant also modifies the diffusion of the active in the liquid crystal. It is widely recognized that liquid crystal structures modify the diffusion properties of materials entrapped within their structures. NMR measurements [43], for example, demonstrate that the structure constricts the degree of self-diffusion. It can be further postulated that diffusion is additionally modified by the inclusion of a charged surface, which causes actives to be held further as demonstrated in inverse chromatography experiments. Taken together, diffusion through cubic phase liquid crystals has been shown to follow a matrix model [44], where release is proportional to the square root of time. It is suggested that the diffusion coefficient in this model is thus dependent on both the matrix and the charge of the surface. The release data redrawn to the square root of time for short times are plotted in Fig. 10. Unlike the work reported by Lindell et al. [36], the slopes of these plots (modified versus unmodified) are not equal, as are the plots reported with DSPG. It is likely that the differences reflect the enhanced association with the DEEDAC and active. It is also possible that the hydrophobic nature of ionized Ketoprofen contributes to this difference (versus Timolol), but this cannot be conclusively deduced from these data. One other important point is that despite the increase in the lattice parameter (Table 2), the gels continue to act effectively to control the release of the ketoprofen. This is particularly good news, as the inclusion of charged surfactant typically increases the magnitude of the lattice parameter [45]. Finally, these data open the possibility of superenhanced loading and release by using, for example, high charge density species such as polyelectrolytes.

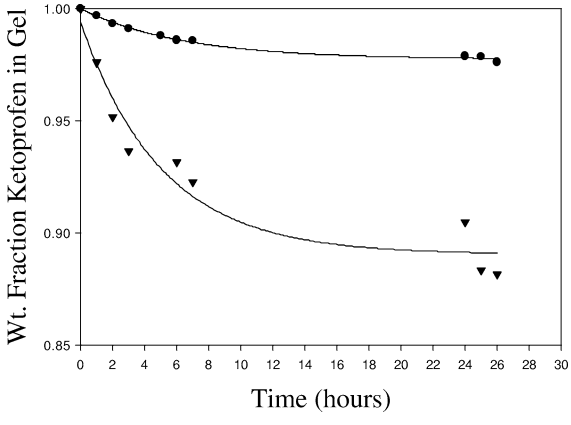


Fig. 9. Controlled release of ketoprofen from functionalized and unmodified cubic phase liquid crystals. The bottom curve shows the release of 2.0 wt% ketoprofen from unmodified cubic phase liquid crystal. The top curve shows the release of 2.0 wt% ketoprofen from a cubic phase liquid crystal modified by the inclusion of 20 wt% of DEEDAC. Evidently the inclusion of the surfactant both slowed the diffusion of the ketoprofen from the phase but also changed the partition.

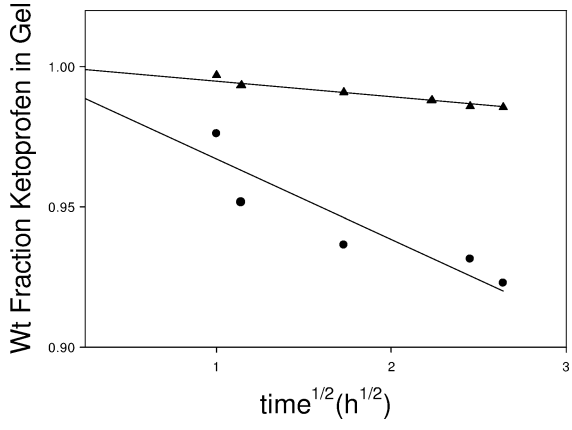


Fig. 10. Release data fit to a matrix model [44]. The concentration of ketoprofen in the gel is plotted versus the square root of time. Both plots show a linear relationship, but with different slopes reflecting the different diffusion profiles of the active.

4. Summary

In considering the use of bicontinuous cubic phase liquid crystals for drug delivery and other applications, there are two valid criticisms of the matrix [16]. First, the liquid crystals are too viscous for many applications. This liability is effectively avoided by the application of existing hydrotropic dilution [46] and powder precursor [47] techniques for preparing cubic phase liquid crystals. Each technique allows fabrication of cubic phase liquid crystalline material while avoiding unduly viscous pathways. Second, the release times for the gels can be too short. Taken in broader context, there is a need to customize all aspects of the properties of the liquid crystal, including release rates. This work demonstrates than cationic surfactants such as DODMAC, DOAC, and DEEDAC can be incorporated into the bicontinuous cubic phase liquid crystals, creating a positively charged matrix that increases the attraction and loading of materials (and effectively lowers the release rate). Further, we have shown how drug uptake can be customized to a far greater extent than previously reported [35].

The enhanced partitioning demonstrated in this work is most effective when the cubic phase is tolerant of significant amounts of charged surfactants. We have demonstrated the necessary criteria for successful surfactant functionalization candidates. However, the application or formulation at hand must also be able to practically and economically accommodate the addition of functionalizing materials in excess of the active material to be delivered. Given the relative cost of surfactant and pharmaceutical active ingredients, the current approach seems quite feasible. The approach we have demonstrated is relevant to both equilibrium loading of active into cubic phase as well as kinetic release of the active material. Further, the charged functionalization groups offer the opportunity for triggered release of the actives as a result of stimuli like pH changes. Future work will address the kinetic performance of such systems.

Acknowledgments

The authors gratefully acknowledge Jeff Grothaus for the SAXS work, Professors Eric Kaler (University of Delaware) and Stig Friberg (Clarkson University) for many useful discussions, and Dave Piatt (the Procter & Gamble Company) for challenging technical input.

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