Formulating Stable Hexosome Dispersions with a Technical Grade Diglycerol-Based Surfactant

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ABSTRACT

We report on the phase behavior of a technical grade and commercially available diglycerol monoisostearate, C41V, and its use for the preparation of nanostructured liquid crystal dispersions (hexosomes). C41V in water forms a reverse hexagonal liquid crystal at room temperature and in a wide range of concentrations (0.5 wt% to 95 wt%); this hexagonal liquid crystal is stable up to 70 °C. A simple and effective method has been developed to disperse hexosomes with an encapsulated active molecule (Ketoprofen) that consist of (1) producing a nano-emulsion stabilized by an amphiphilic block copolymer (Pluronic F127) and containing ethyl acetate and C41V by using ultrasounds and (2) evaporating the solvent to produce

hexosomes. The size of the hexosomes and ultrasound dispersion time is markedly reduced by using ethyl acetate as an auxiliary solvent with an optimal initial ratio of C41V:ethyl acetate of 50:50. Dynamic light scattering shows that the size of the hexosomes decreases as the concentration of stabilizer F127 or encapsulated Ketoprofen is increased. The distance between planes in the hexagonal structure is calculated from small angle scattering data to be ca. 4.6 nm and is only slightly dependent on the amount of F127 and/or encapsulated Ketoprofen. Cryo electron microscopy reveals that the samples contain hexosomes and these coexist with spherical, likely F127 micelles. Lastly, hexosomes show a pH responsive release of Ketoprofen which could be useful for target delivery in the gastrointestinal tract.

INTRODUCTION

Polar lipids can spontaneously self-organize into a variety of well-defined aggregates exhibiting long – range order in two and three dimensions. In this regard, the aqueous phase behavior of polyglycerol-based surfactants has attracted attention in a wide range of fields, from food industry,[1,2] to biotechnology[3] and drug delivery.[4] For example, the phase behavior of glycerol monooleate (GMO) in water or aqueous solution as a function of temperature and composition has been extensively investigated in the last decades.[5–7] Pure GMO forms four distinct liquid crystalline phases that coexist with excess water: Lamellar (L_α), two bicontinuous cubic phases (Q^{230} and Q^{224}), and a reverse hexagonal phase (H_2). Likewise, many hydrophobic polyglycerols fatty acid esters are found to self-organize into reverse liquid crystalline structures.[8,9] Generally, all of these bulk liquid crystalline reverse phases can be dispersed in excess water to produce nanoparticles which retain the internal structure.[10–12] Liquid crystal

nanoparticles (LCN) exhibiting L_α , H_2 and Q internal structures are known as liposomes, hexosomes and cubosomes, respectively. These materials display a high surface area matrix consisting of hydrophilic and hydrophobic regions. Both of these regions can be used to encapsulate a wide range of drugs.[12–14] Moreover, lipid-based LCN can be efficiently degraded in the body by enzymes such as lipase, which is advantageous to prevent bioaccumulation.[15] Liposomes have long been exploited for the transportation and release of a wide range of biopharmaceuticals, ranging from small active molecules to high molecular weight proteins. However, it is not until recent years that hexosomes and cubosomes have been in the spotlight due to their structural robustness and capacity to provide complex diffusion pathways of active compounds.[10,16]

Despite this, there are only few examples of single components that can form different liquid crystalline phases which are stable at room temperatures. For instance, the H_2 phase of pure monoglycerol based surfactants requires high temperatures to form, which is not feasible for many bio-applications.[6,8,9] Therefore, a mixture of two or more lipids is typically used to tune the phase behavior.[17–19] It is also noteworthy that from the industrial point of view, technical grade surfactants are preferred due to their low cost and availability. Commercial technical-grade surfactants are often a complex mixture of amphiphilic molecules with varying chain lengths and esterification degrees. Even though the variability between these mixtures highly influence the self-assembly behavior of the product, only few systematic studies have been carried out on technical grade products compared to those from pure surfactants.[20,21] In fact, the presence of impurities in technical grade surfactants may favor the formation of inverse liquid crystalline phases at room temperature. Examples exists in which technical grade surfactants have been used for preparing LCN.[22–24] Another factor affecting industrial and academic deployment is that

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4 hexosomes and cubosomes are generally prepared by mechanical homogenization, ultrasounds
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6 and/or microfluidification.[25–28] These methods require a high energy input and in most cases
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8 the samples need to be heated. This causes degradation of encapsulated thermolabile substances,
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10 thus restricting the use of hexosomes and cubosomes for encapsulating active molecules. There
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12 are only few examples where a hexosome dispersion is achieved under mild conditions (i.e.
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14 moderate shaking), but these have required a specific formulation.[14,29] On the other hand,
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16 LCN can be obtained with considerably less energy by using a hydrotome [30–33]. More
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18 importantly, these methods have proven to be suitable for preparing large volumes of LCNs.[31]
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25 Whilst the above examples clearly highlight the potential of LCNs, their applications are still
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27 relatively scarce. It is therefore necessary to expand the library of surfactants forming LCNs and
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29 improve the preparation methods. Herein we present a study on the phase behavior as a function
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31 of temperature and composition of a technical grade surfactant, diglycerol-monoisostearate. This
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33 surfactant forms a reverse hexagonal liquid crystal in excess water at room temperature. A
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35 straightforward method was developed to produce hexosomes with encapsulated active
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37 molecules by using solvent evaporation with nano-emulsions as templates and a block copolymer
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39 as stabilizer. Nano-emulsions restructure into hexosomes prior removal of the auxiliary solvent
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41 (ethyl acetate). The size of the particles depends on stabilizer concentration, initial amount of
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43 solvent and concentration of encapsulated drug.
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50 **EXPERIMENTAL**

51 **Materials**

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54 Technical grade diglycerol mono-isostearate surfactant (**C41V**, Scheme 1) was a gift from the
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56 Nisshin OilliO Group, (Ltd., Japan). The main impurities consist of diglycerol isostearates with
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different degrees of esterification (from 1 to 4) and different chain length fatty acids. The surfactant was used without further purification. Pluronic® **F127** (BASF, Germany) was used as a stabilizer. Ethyl Acetate (**EAc**, 99.7 %, Merck) was used as volatile organic solvent. Millipore-filtered water was used in all experiments. Simulated gastric media without enzymes from saline concentrate (pH= 1.3, Sigma-Aldrich) and PBS (136.89 mM NaCl, 0.93 mM NaH₂PO₄·H₂O and 16.76 mM Na₂HPO₄·2H₂O, Sigma-Aldrich) were used as receptor solution for the drug release experiments.

Scheme 1. Molecular structure of diglycerol mono-isostearate surfactant.

Methods

Phase Diagram Determinations.

Samples with different water/**C41V** ratios were sealed in glass tubes with a narrow constriction. Viscous samples were homogenized by repeated centrifugation. The phase boundaries were detected by birefringence changes using Polarized Optical Microscopy (**POM**) with an Olympus BX51TRF6 microscope. Temperature was controlled in the 10 – 80 °C range using a Peltier hot stage with a precision of ± 1 °C.

Small-angle X-ray Scattering (SAXS).

SAXS measurements of high concentration samples were performed in an S MICRO instrument (Hecus X-ray System, Graz, Austria) with point focalization, and equipped with a GENIX

microfocus X-ray source in 1 mm quartz capillary tubes sealed with wax. The scattering intensity was recorded as a function of the scattering angle, q .

$$q = \frac{4\pi \sin(\theta)}{\lambda} \quad (1)$$

Where λ is the radiation wavelength (1.54 Å) and θ is the scattering angle. Measurements were performed with the X-ray source at 50 kV and 1 mA. The exposure time for measurements was 30 minutes for concentrated samples. The lattice parameter, a , for a hexagonal liquid crystal is defined as

$$a = \frac{4\pi}{q\sqrt{3}} \quad (2)$$

SAXS experiments of low-concentration dispersions were carried out in the beamline NCD11 at ALBA Synchrotron Barcelona (Spain). The energy of the incident beam was 12.6 KeV ($\lambda = 0.995$ Å). Samples were placed in wax-sealed quartz capillaries (Hilgenberg, Germany) with an outside diameter of 1.5 mm and wall thickness of 0.01 mm. The scattered X-ray was detected on a Pilatus 1M detector (Dectris), converted to one-dimensional scattering by radial averaging, and represented as a function of q . Data on each sample were collected over 60 s.

Hexosome Preparation

C41V (0.5 g) was dissolved in **EAc** (ratios **C41V:EAc** of 1:0, 7:3, 5:5 and 3:7). 9.5 g of a solution containing **F127** in water or phosphate buffer (to match **C41V:F127** ratios of 9:1, 8:2, 7:3, 6:4) were added to the solution of **C41V** in **EAc**. The mixture was vortexed for 1 minute. The resulting emulsion was further dispersed with an ultrasonic probe (KE-76 probe, Bandelin

Sonoplus, operating at 20 kHz and 100 μm peak-to-peak amplitude). **EAc** was removed in a rotary evaporator for 1.5 h at 25 °C under reduced pressure.

Particle Size Determinations

Particle size was determined by dynamic light scattering (DLS) on a 3D DLS spectrometer (LS instruments, Switzerland) equipped with a 35 mW He-Ne laser light source ($\lambda = 632.8 \text{ nm}$). The instrument uses two lasers and cross-correlates both signals to suppress multiple scattering. The samples are placed in 5 mm cylindrical borosilicate tubes, kept in a 25 °C bath with a refractive-index-matching liquid (decalin), and detected at a scattering angle of 90 °. The decay rate (derived from the second order cumulant analysis of the normalized autocorrelation function) is used to calculate the mean diffusion coefficient, D of the particles. The hydrodynamic radius, R_H , was calculated assuming spherical morphology using the Stokes-Einstein equation:

$$R_H = \frac{k_B T}{6\pi\eta D} \quad (3)$$

where k_B , T and η are the Boltzmann's constant, absolute temperature and dynamic viscosity of water, respectively. The error was calculated from the standard deviation of 3 different experiments as:

$$error = \frac{SD}{\sqrt{n}} \quad (4)$$

Where SD and n are the standard deviation and the number of experiments, respectively.

Cryo-Transmission Electron Microscopy

Vitrified specimens were prepared in the Vitrobot (FEI) at a controlled temperature of 25 °C and water saturation. Specimens were examined in a FEI Talos F200C transmission electron microscope (TEM) at cryogenic temperatures. Images were recorded on Falcon II™ direct electron,[34] at low dose conditions.[35] Volta Phase Plate (VPP) was used for contrast enhancement.

Ketoprofen Encapsulation and Release

The hexosomes loaded with Ketoprofen (**KP**) were prepared by dissolving the drug in the organic phase (**C41V:EAc**) before adding the **F127** aqueous solution and then following the procedure described above. **HPLC** was used to quantify the amount of **KP** released from a sample to a receptor solution using acetonitrile as an organic phase and a solution of Citric acid (7.3 g/L), NaCl (3.8 g/L) and NaOH (0.15 g/L) (Organic:Aqueous ratio of 55:45). 20 µL of sample was injected into a C18 column with a flux of 1 mL/min. The retention time at these conditions was 4.5 minutes.

Thermo-jacketed glass vessels were used to maintain the desired temperature (37 °C) during the whole release. They were filled with 40 mL of two receptor solutions: PBS and simulated gastric fluid. Spectra/Por Float-A-Lyzer G2 (Spectrum Labs) devices with a membrane composed of Biotech Grade Cellulose Ester with a molecular weight cut-off of 3.5 – 5 kDa were pre-wetted with 10 wt% EtOH in water for 20 minutes followed by a second wetting step of distilled water. They were then filled with 2 mL of sample and soaked into the receptor solution. Aliquots of 1 mL of the receptor solution were withdrawn from the experimental setup at different time intervals for analysis (4 replicas per sample). The amount of **KP** was calculated so that the final

concentration in the receptor solution was 20 wt% of the total **KP** saturation concentration in that media (Sink Conditions). The error bars were calculated with equation (3) from 3 different experiments. The release profiles were fitted to the Korsmeyer-Peppas model:

$$C = K_{KP} t^n \quad (3)$$

where K_{KP} is a kinetic constant characteristic of the system, and n is an exponent that depends upon the release mechanism.

RESULTS AND DISCUSSION

The phase diagram of the water/**C41V** system, determined as a function of temperature and **C41V** concentration, was built based on macroscopic and microscopic observations (Figure 1a). The water/**C41V** system is characterized by two multiphase regions: one containing hexagonal liquid crystals at **C41V** concentrations from 0.5 wt% to 95 wt% and below 70 °C and the other above 70 °C consisting in two liquid phases. Samples in the concentration range between 50 wt% and 95 wt% at 25 °C display high viscosity and turbidity. A visible macroscopic phase separation between a turbid, viscous liquid and water can be observed in samples with **C41V** content below 40 wt%. This is in accordance with other reports showing that diglycerol based surfactants containing impurities stabilize a lamellar phase in excess water at room temperature. These impurities are mainly unreacted diglycerol and diglycerol fatty acids with different degrees of esterification.[20] In our case the absence of a monophasic region is ascribed to the presence of impurities.

Figure 1. (a) Binary phase diagram of **C41V** in water as a function of temperature and concentration. The discontinuous line depicts the boundary at which a macroscopic phase separation is observed. (b) **POM** of a 50 wt% sample showing a fan-shaped conic texture characteristic of hexagonal liquid crystals.

In the multiphase region all samples present strong backscattering (turbidity). Birefringent samples display a fan-shaped conic texture under **POM** (Figure 1b), consistent with a hexagonal liquid crystal. A reverse hexagonal liquid crystal (H_2) is deduced considering the long hydrophobic chain of **C41V**, which favors a spontaneous negative curvature of the system.

Samples at temperatures higher than 70 °C (up to 80 wt%) showed a macroscopic phase separation into two isotropic liquid phases. The biphasic region resulting from the melting of the H₂ phase consists of a lipidic phase in excess water (Water + L₂). SAXS patterns of samples with **C41V** content up to 95 wt% at 25 °C presented three peaks with the q ratios 1: $\sqrt{3}$:2 assigned to a hexagonal phase (See Supporting Information Figure S1). Samples with **C41V** content higher than 95 wt% showed a broad band, tentatively attributed to a reverse micellar phase (L₂). This micellar phase was not further studied.

The lattice parameter, a , (calculated from the first order peak of the SAXS patterns) increases with water content due to swelling of the H₂ aqueous channels (Figure 2). The maximum swelling of the system is reached at a water content of 60 wt%. Above this, a H₂ phase coexists with excess water. The maximum swelling boundary corresponded to a macroscopic phase separation.

Figure 2. Lattice parameter a calculated from the first SAXS peak as a function of surfactant concentration for the water/**C41V** system at 25 °C. The dotted line indicates the maximum swelling capacity, i.e. macroscopic phase separation.

Formation of hexosomes

The formation of a stable reverse hexagonal phase in coexistence with water can be exploited to form dispersions of these liquid crystals, the so-called hexosomes. Generally, high molecular weight block copolymers can be added to hexosomes to facilitate dispersion and increase colloidal stability by providing a hydrophilic shell that prevents aggregation. Pluronic **F127** is one of the most used agents for dispersing and stabilizing non-lamellar liquid crystal dispersions. The incorporation of **F127** into reverse liquid crystals is expected to be relatively low due to the high molecular weight and hydrophilicity of this polymer. Therefore, the internal structure of the particles can be better preserved within a well-defined range of **F127** concentrations. Generally, a high-energy input is usually required to form hexosomes. For example, ultrasounds are one of the most efficient methods used. During this process, the higher the natural cohesive forces acting within a liquid (e.g. high viscosity and high surface tension) the more difficult is to attain cavitation and thus, more energy is required to break the droplets or particles. Consequently, ultrasonic dispersion is more efficient as the viscosity of the dispersed phase is reduced. In an attempt to reduce the energy spent in the dispersion process, the low toxicity solvent ethyl acetate (**EAc**) is mixed with **C41V** to avoid the formation of the viscous H_2 phase. This mixture was dispersed in a **F127** aqueous solution using ultrasounds, resulting in an Oil-in-Water (O/W) emulsion. Subsequent removal of **EAc** results in reorganization of the surfactant molecules into a hexagonal phase dispersion. As expected, through a combination of ultrasound and the solvent evaporation methodology, the particle size is drastically reduced over a significantly shorter timeframe compared to the dispersion using solely ultrasound under equivalent conditions (see Figure 3).

Figure 3. Hydrodynamic radius, R_H , of hexosomes prepared by ultrasound + solvent evaporation method and solely ultrasound for a sample with a **C41V:F127** ratio of 80:20.

The effect of initial **C41V:EAc** ratio was studied at different **C41V:F127** ratios and 5 wt% of **C41V** dispersed for 2 minutes under ultrasounds. The size of hexosomes decreases with initial **C41V:EAc** ratios until reaching an almost constant value at **C41V: EAc** =50:50 (Figure 4).

Figure 4. Hydrodynamic radius, R_H , of hexosomes as a function of the **C41V** concentration in samples with various **C41V:F127** ratios.

As the ratio of **C41V:F127** is decreased, the particle size is reduced (See Table 1). The reduction of size implies that **F127** arranges on the surface of the liquid crystal particles, reducing the surface tension between the dispersed and continuous phase. At **C41V:F127** ratios higher than 80:20 the size variation with **F127** concentration reaches a plateau. The extra **F127** may form micelles which coexist with the hexosomes, as is previously reported with other systems.[36] Generally, the obtained dispersions have a polydispersity index (PDI) between 0.2 and 0.3.

The size of the hexosomes was slightly smaller when prepared in PBS (Table 1). This effect may be caused by the presence of electrolytes which reduces the solubility of ethyl acetate in the aqueous phase.[37] This would aid in stabilizing the system against Ostwald ripening, which may be significant at early stages of the dispersion. Another effect that should be taken into account is that the presence of electrolytes has been reported to reduce the surface tension in water/surfactant/oil systems,[38] which favors the formation of smaller droplets. The dispersions obtained in PBS presented improved PDIs (<0.2) than those prepared in water.

Table 1. R_H of hexosome dispersions with different **C41V:F127** ratios prepared in water and PBS. The PDI value is between 0.1 and 0.3.

C41V:F127	R_H in Water (nm)	R_H in PBS (nm)
9:1	85.4 ± 3.0	78.3 ± 0.4
8:2	66.1 ± 0.8	64.2 ± 0.2

7:3	67.1 ± 0.4	56.0 ± 0.4
6:4	69.2 ± 1.2	58.1 ± 0.4

Samples could be prepared with up to 20 wt% of **C41V** whilst maintaining the same **C41V:F127** ratios. The particle size is smaller as the total surfactant content is increased (see support information Figure S2). This effect could be caused by the higher concentration of **F127**, suggesting a greater degree of association with the hexosome surface.²⁷ Samples with **C41V** concentrations higher than 5 wt% creamed after a few hours. However, the stability can be enhanced by diluting the dispersions to 5 wt% of **C41V**.

Another factor to be considered is whether **F127** affects the inner structure of the hexosomes. To explore this effect, the influence of **F127** content on the H_2 structure was studied in hexosome samples containing 5 wt% of **C41V** with different **C41V:F127** ratios (Figure 5). Increasing the **C41V:F127** ratio from 100:1 to 90:10 causes the lattice parameter, a , decreases slightly from 54 Å to 52 Å, indicating that **F127** induces a dehydration of the inner hydrophilic channels of the hexosomes, consistent with other reports.[39] This dehydration also seems to increase the crystalline domain size compared to the sample without **F127**, as observed by the more pronounced peaks. With a further increase in **C41V:F127** ratio, the separation between the inner H_2 inner channels increases and the crystalline domains are reduced in size resulting in broader bands. The internal structure of the particles is disrupted at **C41V:F127** ratios of 60:40, which is inferred by the lack of second and third order scattering peaks, as well as broadening of the first order scattering peak.

Figure 5. SAXS patterns of hexosome samples prepared by the ultrasounds + solvent evaporation method containing different **C41V:F127** ratios, an initial **C41V:EAc** ratio of 50:50 and a total surfactant concentration of 5 wt%.

To visually confirm the presence of H_2 and elucidate the morphology of hexosomes, representative samples were examined by Cryo-TEM (Figure 6). The inner hexagonal structure of the particles can be clearly observed. Particle sizes appear to be within the same order of magnitude of those estimated by DLS. It can also be observed that small spherical, likely F127 micelles, coexist with the hexosomes. These micelles become visible due to the synergy of contrast enhancement by the use of the Volta Phase Plate and improved resolution and lower dose through the use of the sensitive Falcon direct electron detector. The lattice parameter was estimated from Cryo-TEM images to be ca. 50 Å, which is in good agreement with SAXS values (52 Å).

Figure 6. Cryo-TEM images of hexosome dispersions of sample of 5 wt% of **C41V** and **C41V:F127** ratio of 70:30. The inset is a magnified area. The red arrows indicate the presence of small, uniformly spherical, likely **F127** micelles.

Ketoprofen encapsulation and in-vitro release

The lipophilic nature of the hexosomes facilitates the solubilization and controlled release of many small active compounds. Ketoprofen (**KP**) is a hydrophobic drug used in the therapy of rheumatic chronic disorders. **KP** has a low bioavailability due its sparing solubility in water, therefore it is a good candidate for encapsulation and release studies. As described above, solubilized additives can influence in the internal structure of the hexosomes. Such changes were assessed by SAXS after the addition of **KP** (Figure 7a). The hexagonal structure was preserved for **KP** contents up to a maximum of 1 wt% (**C41V:KP** = 5:1). At higher concentrations, excess **KP** precipitated. The lattice parameter also increases with **KP** content, indicating the incorporation of **KP** into the hydrophobic regions of the hexosomes (inset Figure 7a). The size of the hexosomes is also reduced with increasing concentration of **KP** (Figure 7b). Similar to the

effect of cholesterol on lipid bilayers, **KP** may reduce the viscosity of the H_2 phase by reducing short range alkyl-alkyl interactions and thus favoring the formation of smaller hexosomes.[40] Furthermore, **KP** can also increase the long range order, in agreement with more pronounced peaks with increasing **KP** concentration.[41,42]. Reports also show **KP** can reduce the interfacial tension,[43] therefore, **KP** may also be located both at the surface and in the lipophilic regions of the hexosomes. Significantly, the size of the hexosomes (determined by DLS) remained constant for at least 3 months, illustrating their remarkably high stability.

Figure 7. (a) SAXS patterns of hexosome samples (5 wt% **C41V** and **C41V:F127** = 80:20) with different amounts of encapsulated **KP**. The change in the lattice parameter a with **KP** concentration is shown in the inset (b) hydrodynamic radius of the hexosomes samples as a function of **KP** concentration.

KP encapsulation efficiency was studied by the indirect filtration-centrifugation method. A sample containing 0.5 wt% of **KP** encapsulated in 5 wt% **C41V** with **C41V:F127** ratio of 80:20 was chosen as the optimal formulation as it favorably achieves high stability, small hexosome size, and reduces the concentration of **F127** whilst maintaining a high degree of drug loading. **HPLC** analysis was performed to determine the amount of drug in the aqueous continuous phase. About 86 % of drug was encapsulated in the hexosomes.

Simulated gastric fluid (pH=1.2) and PBS (pH=7.4) were used as receptor solutions. As seen in Figure 8, complete release of free (non-encapsulated) **KP** was achieved after 9 h irrespective of receptor solution pH, indicating that **KP** could diffuse freely through the dialysis membrane. As expected, encapsulated **KP** showed a slower release as compared to free **KP**. During the first 9 h, the release profile is roughly equivalent at pH = 1.2 and 7.4. Complete release of **KP** to PBS (pH=7.4) was achieved after 25 hours, whereas the release from hexosomes to simulated gastric fluid (pH=1.2) was about X % in the same period.

Figure 8. Release profiles of **KP** from an aqueous solution, and from a hexosome dispersion with 5 wt% of **C41V** and a **C41V:F127** ratio of 80:20 to receptor solutions at different pH and at 37 °C. Lines are only a visual guide.

The experimental datasets were fitted to the Korsmeyer-Pepas model for total drug release values lower than 60 % to obtain the diffusional exponent, n (see support information, Figure S3). **KP** aqueous solutions presented an n value close to 0.5, characteristic of a release mechanism under Fick's regime in which release is dominated by diffusion. In contrast, for the encapsulated **KP** the n values were *ca.* 0.8, indicating a non-Fickian transport in which the release mechanism is governed by diffusion and swelling of the hydrophilic nano-channels. Indeed, the slow rearrangement of hydrophobic tails when the local concentration of **KP** in the lipophilic region is reduced may cause the non-Fickian behavior. The lower amount of **KP** released at long-times and low pH may arise from a decreased solubility of **KP** in acidic media.

Conclusions

The phase behavior of the water/**C41V** system as a function of **C41V** concentration and temperature is characterized by a wide multiphasic region with the presence of inverse hexagonal

liquid crystal (H_2) at temperatures below 70 °C. The absence of a monophasic H_2 region was ascribed to the presence of impurities. This H_2 phase in the water/**C41V** system can be dispersed in excess water in the form of nanoparticles stabilized by **F127**. We introduce an adapted solvent evaporation method to produce hexosome dispersions using ethyl acetate as a volatile solvent, followed by ultrasonic dispersion and further solvent evaporation. With significantly reduced energy input we produced hexosome dispersions with small droplet size and achieved greater control over polydispersity. Our dispersion approach reduces considerably the amount of energy needed to obtain the dispersions, compared to conventional methods reported in for example references [22–25]. The size of the hexosomes can be tuned by varying the **C41V:F127** ratio, the initial **C41V:EAc** ratio and salt concentration. The hexagonal structure of the particles do not change upon addition of **F127** (up to **C41V:F127** ratios of 70:30) indicating that the stabilizer is located at the interface between hexosome and water. Cryo-TEM confirmed the coexistence of hexosomes with **F127** micelles, concomitant with our experiments and recent literature reports.[31 **C41V:KP** ratios of up to 5:1 could be encapsulated into the dispersions without perturbing the internal structure of the hexosomes. Controlled-release experiments showed that 60 % of total encapsulated **KP** is released within the first 9 hours via non-Fickian transport. The prolonged **KP** release rate to receptor solutions is slower at low pH which is likely due to a decrease in **KP** solubility. The present work can be applied generally for the preparation of stable liquid crystal dispersions. This will eventually increase the interest of these soft-systems to industry for developing new applications in the market.

ASSOCIATED CONTENT

Supporting Information. Concentration dependent SAXS patterns from the C41V/water phase behavior; Hydrodynamic radius of hexosomes samples as a function of C41V content; Fitting lines of release experimental data to the model of Korsmeyer-Peppas.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

GMO, glycerol monooleate; H₂, inverse hexagonal liquid crystal; LCN, liquid crystal nanoparticles; F127, pluronic F127; EAc, ethyl acetate; PBS, phosphate buffer; POM, polarized optical microscopy; SAXS, small-angle X-ray scattering; DLS, dynamic light scattering; Cryo-TEM, cryo-transmission electron microscopy; KP, Ketoprofen; HPLC, high pressure liquid chromatography.

KEYWORDS

Phase Behavior; Surfactant; Hexosome; Liquid Crystal Nanoparticle; Drug-release

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TOC graphics.