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Lyotropic liquid crystalline phases formed from glycerate surfactants as sustained release drug delivery systems

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Abstract

A new class of surfactants with glycerate headgroups, that form viscous lyotropic liquid crystalline phases in excess water, have been investigated for their potential to provide sustained release matrices for depot drug delivery. Oleyl glycerate and phytanyl glycerate were used as representative surfactants of this new class, and their behaviour compared with that of glyceryl monooleate (GMO). The surfactants were found to form reverse hexagonal phase (H_{II}) in excess water, and the matrices were loaded with a series of model hydrophobic and hydrophilic drugs, (paclitaxel, irinotecan, glucose, histidine and octreotide), and the release kinetics determined. In all cases, the release behaviour obeyed Higuchi kinetics, with linear drug release versus square root of time. The H_{II} phases released model drugs slower than the GMO cubic phase matrix. The oleyl glycerate matrix was found to consistently release drug faster than the phytanyl glycerate matrix, despite both matrices being based on H_{II} phase. To further demonstrate the potential utility of these materials as drug depot delivery systems, an injectable precursor formulation for octreotide was also prepared and demonstrated to provide controlled release for the peptide. The stability of the H_{II} phase to likely in vivo breakdown products was also assessed.

Keywords: Parenteral depot formulations; Cubic phase; Hexagonal phase; Glycerate surfactant; In vitro release

1. Introduction

Sustained release of parenterally administered drug products is often a desired therapeutic outcome as it permits fewer injections, increases patient compliance and may reduce side effects, compared to simple solution or intravenous administration. There are a number of platform technologies currently used in marketed drug products that provide slow release from the subcutaneous, intramuscular and intraperitoneal tissue. Implantable devices aside, the syringable platform technologies which form the basis of these products primarily include oily suspensions, polymer-based gels and polymer-based microparticles. The polymer-based systems utilise biodegradable poly(lactic acid) or poly (lactide-co-glycolide) copolymers.

Suspensions and oily solutions have been the mainstay of sustained release depot injections for decades, but these delivery systems are less well suited to water soluble drugs, are generally are often painful on administration leading to poor patient compliance, and such products frequently also suffer from poor stability and variable absorption kinetics. Whilst polymer-based solution or gel-type systems provide sustained release over the order of months, they require the drug to be dispersed or dissolved in the same solvent used to dissolve the polymer for administration (e.g. *N*-methyl pyrrolidone) (Brodbeck et al., 1999).

Parenteral dosage forms of hormone regulating peptides, proteins and antibodies are almost always required as these therapeutic agents are often unstable in and/or poorly absorbed from the gastrointestinal tract. Sustained release platforms are particularly attractive for such agents due principally to their often short biological half lives supported by an increasing trend towards improved sustained release formulations for old drugs.

The polymer-based technologies have provided a number of currently marketed long acting depot products, including Sandostatin LAR® (containing octreotide acetate for acromegaly), Risperdal Consta® (containing the antipsychotic, risperidone),

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Eligard® (containing luteinising hormone releasing hormone or LHRH) and Nutropin® (containing recombinant human growth hormone). The major drawback with PLGA-based systems is the need for either a solvent, such as *N*-methyl pyrrolidone to dissolve the polymer and drug, or the need to prepare microspheres of polymer encapsulating the drug, which can be technically difficult to manufacture and sterilize, and may cause problems at the time of injection. One alternative formulation platform to the PLGA systems is the SABER® (sucrose acetate isobutryate) technology, however this system also has the drawback that a solvent is required to disperse the components, and it also exhibits significant burst release unless appreciable levels of PLA is added to the formulation (Okumu et al., 2002). Polymeric microsphere suspensions are difficult to manufacture and sterilise and, anecdotally, polymeric microsphere technology, whilst avoiding the use of harsh solvents in the injected product, can be difficult to administer in the clinical setting due to the relatively large particle to needle size ratio.

Other lipid-based depot formulations, based on phospholipids, include Supravail® which is particularly suited to poorly water soluble drugs and SkyePharma's DepoFoam™, a network of liposome-like compartments which forms the basis of the marketed DepoCyt® cytarabine injectable depot product (Asherman et al., 1998). This product is supplied as an aqueous dispersion/suspension, and consequently when applied to drugs other than cytarabine may encounter stability problems on long-term storage in an aqueous environment.

It is clear that there are limitations with each of the available technologies, and that a new platform technology that could overcome some of these limitations would be desirable.

It is known that viscous reverse lyotropic liquid crystalline phases in excess water, such as reverse hexagonal and bicontinuous cubic phase, can provide a slow release matrix for incorporated active molecules (Drummond and Fong, 1999; Shah et al., 2001). These materials provide a complex matrix consisting of discrete aqueous and lipidic regions. They also possess a high internal interfacial area, estimated to be in excess of 400 m^2/g (Lawrence, 1994). This heterogeneous structure permits incorporation of hydrophilic, lipophilic and amphiphilic materials, or a combination thereof, within the limitation that their presence does not induce a change in lyotropic phase structure (Mueller-Goymann and Frank, 1986). Judicious choice of materials to form the liquid crystal system can provide a matrix that is stable to dilution on contact with bodily fluids, which in principle could form a persistent, sustained release depot when administered by any number of routes to the patient.

Until recently there have been few materials identified that have been capable of forming such liquid crystalline matrices in excess aqueous solution; research has primarily utilised glyceryl monooleate (GMO) and mixtures of GMO with other lipids, or variants on the GMO structure (Qui and Caffrey, 1998). Minamikawa and Hato have reported glycosides that exhibit this type of phase behaviour (Minamikawa and Hato, 1997). Phytantriol (3,7,11,15-tetramethyl hexadecantriol) also forms a cubic phase (Q) in excess water at ambient and physiological temperatures (Barauskas and Landh, 2003), and has been receiving more attention recently as it is commercially available, and with a phytanyl backbone provides important structural differences to the fatty acid-based materials.

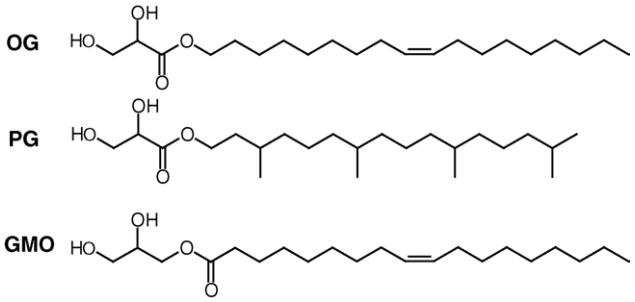

Fig. 1. Chemical structures of the three surfactants used to form liquid crystalline matrices, OG: oleyl glycerate; PG: phytanyl glycerate and GMO: glyceryl monooleate.

We recently disclosed a new class of lyotropic liquid crystal forming materials with glycerate headgroups (Boyd et al., 2004, 2005). Importantly, it was discovered that oleyl glycerate (OG, 2,3-dihydroxypropionic acid octadec-9-enyl ester) and phytanyl glycerate (PG, 2,3-dihydroxypropionic acid 3,7,11,15-tetramethyl-hexadecyl ester) are able to form reverse hexagonal phase (H_{II}) at physiological temperatures in excess water. The structures of OG, PG and GMO are presented in Fig. 1. The very subtle regio-chemical difference in structure between OG and GMO induces interesting differences in their self-assembly properties. The discovery of these materials has further expanded the pool of surfactants known to form cubic and reverse hexagonal phases at physiological temperatures, which may subsequently be utilised as the basis of sustained release delivery systems in vivo.

In this report, we detail the phase behaviour and formulation aspects of oleyl glycerate and phytanyl glycerate as a liquid crystal-based depot injection for both hydrophilic and hydrophobic drugs. Two hydrophobic model drugs were chosen for these studies: paclitaxel, and irinotecan in base and hydrochloride form (IrB and IrHCl, respectively), and one hydrophilic drug, octreotide acetate, a peptide with molecular weight of approximately 1.5 kDa. Glucose and histidine were also studied as representatives for uncharged and charged, smaller model hydrophilic molecules, respectively. Preparation of injectable precursor forms of the mesophases for delivery of hydrophilic drugs has been achieved by the formulation of low viscosity, water-containing precursors of the lyotropic phases, by taking advantage of their lyotropic phase behaviour. These precursor systems form the liquid crystalline phase in situ, and may provide a clinically useful controlled release alternative. Stability of the liquid crystalline phase to likely breakdown products of the lipids in the form of hydrolysed fatty alcohols has also been assessed through investigations of phase behaviour at various levels of added alcohol. Release of the model drugs from the liquid crystalline matrices and from precursor formulations has been determined in vitro and compared to equivalent GMO-based systems.

2. Materials and methods

2.1. Materials

OG and PG were manufactured at CSIRO-Molecular Science (Australia) as disclosed previously (Boyd et al., 2004) and were (99%) and (96%) pure by reverse phase HPLC, respectively. Myverol 18-99^{TM} (Myverol) was obtained from Kerry Bio-Science (Norwich, NY). Myverol has a high monoglyceride content and is known to have very similar phase behaviour to pure glyceryl monooleate (Clogston et al., 2000), most importantly that it forms cubic phase in excess water at physiological temperatures, and therefore is a good model for GMO. Paclitaxel was obtained from Napro Biotherapeutics, Colorado, USA. Irinotecan base ((*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3′,4′:6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4′bipiperidine]-1′-carboxylate) and hydrochloride were obtained from Dabur, India and were used as received. Octreotide acetate was obtained from Lipotec, Barcelona, Spain. Oleyl alcohol and phytanol were obtained from Sigma (Sydney, Australia).

2.2. Solubility of model drugs in surfactants

The solubilities of irinotecan base, irinotecan hydrochloride and paclitaxel in Myverol, PG and OG, were determined by equilibration of excess drug in the surfactant in triplicate at 37 °C, centrifuging the samples and removing a sample of the drug-free supernatant for assessment of drug content by HPLC. Samples were taken over several weeks every three days until there was less than 5% change in drug content between sampling times.

2.3. Phase behaviour studies

Phase behaviour studies were conducted to determine (i) the phase progression displayed by the lipid with increasing water content, (ii) the water content at the boundary of the lyotropic liquid crystalline phase in contact with excess water, (iii) what influence dissolved drug has on the phase progression displayed by the lipid with increasing water content and (iv) what influence the corresponding fatty alcohol has on the phase behaviour as an indicator of in vivo hydrolysis of the glycerate surfactant by esterase activity. Phase progressions were determined by flooding experiments conducted using crossed polarised optical microscopy on a Zeiss Axiovert Inverted microscope (Carl Zeiss, Melbourne, Australia), fitted with a Mettler TA3000 hot stage (Mettler Toledo, Melbourne, Australia). The surfactant, surfactant + fatty alcohol, or surfactant + drug was placed between a microscope slide and coverslip and flooded with water at 37 °C, and the birefringent textures observed at the excess water boundary. In order to determine the water content at the reverse micellar (L_2) to reverse hexagonal phase boundary, required for the formulation of low viscosity precursor solutions, samples of increasing water content were prepared and equilibrated as detailed below for coulometry measurements, and the appearance of birefringence when the sample was viewed through crossed polarising filters (Rosevear, 1968), and a visual decrease in sample flowability taken to indicate the onset of H_{II} phase formation.

To determine the water content at the excess water boundary, Karl Fisher coulometry, conducted on a Metrohm KF 684 coulometer (Mettler Toledo, Melbourne, Australia) was utilised. Triplicate samples of the surfactant or surfactant/lipid mixtures (∼400 mg) were intimately mixed with a large excess of water, by rapidly vortexing for 30 s at 80 °C (where low viscosity L_2 phase exists) and immediately cooling back to 37 °C. The heat/vortex/cool cycle was repeated three times and the sample stored at 37 °C for 100 h before analysis and visually observed for uniformity between crossed polarising filters. Prior to analysis, the sample was centrifuged and excess water removed. Small samples (∼20 mg) were removed from different sections of the liquid crystalline matrix, and dissolved in acetonitrile for injection into the coulometer. The water content of an equivalent volume of blank acetonitrile was subtracted from the measured value of the water content, and the weight of the samples used to calculate the percentage of water content in the original sample.

2.4. Formulation of precursor and pre-formed liquid crystalline systems

Formulations of the pre-formed liquid crystalline phase were prepared containing 25% water to ensure minimal water uptake on immersion in the release medium. The sample was mixed and stored at 37 °C for 100 h prior to use as described in the coulometry experiments. For the hydrophobic drugs, the drug was dissolved at saturation levels in the surfactant prior to addition of the aqueous phase, whilst the hydrophilic drugs were dissolved in the aqueous phase prior to addition to the surfactant. The injectable precursor solutions were prepared by dissolving the hydrophilic drug in the aqueous solution and adding the aqueous solution to the surfactant such that the water content was only 7% (w/w), thereby ensuring the existence of the low viscosity L_2 phase.

2.5. Drug release from pre-formed liquid crystalline systems

Drug was loaded into the bulk lyotropic phase by either dissolving the drug in the surfactant or aqueous component, depending on whether the drug was lipophilic or hydrophilic, respectively. The lipid phase, with or without drug, was placed in a sealable glass vial (generally ∼300–400 mg) and the aqueous phase (with or without drug) was placed on top of the lipid phase (500–1000 mg) to form the lyotropic phase. The sample was agitated at 37 °C by means of a tube roller for 4 days to ensure equilibration of the bulk lyotropic phase, confirmed by crossed polarised optical microscopy.

A sample of the bulk lyotropic phase was then removed and placed into a small purpose-built glass microbeaker with approximately 500 mg capacity, which was anchored to a large magnetic stirrer to prevent floating of the microbeaker in the release medium. The microbeaker was then immersed in a thermostatted beaker at 37 °C containing the release medium (typically 500 mL

of MilliQ water), and stirred by means of a digital overhead propeller stirrer (Eurostar) at 100 rpm (30 mm tri-prop stirrer shaft). The release medium was sampled periodically (100 μL) and replaced with fresh release medium. The samples were analysed for drug content by HPLC. As many of the experiments were not conducted strictly under sink conditions, samples were generally taken until a plateau had been achieved to allow relative release rates to be compared from the different surfactant–drug combinations.

Drug release has been plotted against the square root of time, as drug release from mesophase matrices has been shown to be primarily controlled by diffusion through the matrix and consequently can be described by the Higuchi diffusion equation given by:

$$Q = [D_m C_d(2A - C_d)t]^{1/2}$$

where Q is the mass of drug released at time t, and is proportional to the apparent diffusion coefficient of the drug in the matrix, D_m the initial amount of drug in the matrix, A and the solubility of the drug in the matrix C_d (Higuchi, 1967). The slope of the linear fit to the data from this plot is proportional to the apparent diffusion coefficient for the drug in the matrix, and permits firstly, assessment of diffusion as the primary means of drug release from the correlation coefficient for the linear fit, and second, a means to compare the diffusion of a drug from the different matrices into the release medium.

2.6. Drug release from injectable precursor systems

The release of octreotide acetate from the precursor system was conducted in a 50 mL polypropylene tube. Due to the propensity of the L_2 phase to floating, and thereby sticking to the wall of the tube, the L_2 phase was prepared as described above and injected into a soft gel capsule. The soft gel capsule was then placed into the release medium in the tube on a shaking water bath at 37 °C, where the capsule rapidly dissolved, permitting formation of a single mass of lyotropic liquid crystal. Samples (100 μL) of the release medium were removed and analysed for drug by HPLC, and the volume replaced with fresh release medium.

2.7. Analytical methods

Drug content in surfactant samples and release media was determined by HPLC methods. In all cases except for glucose an Altima C8 5 μm, 250 mm × 4.6 mm column (Alltech, Melbourne, Australia) was employed. For glucose, a Phenomenex Luna NH_2 column, 250 mm × 4.6 mm, 5 μm column was used (Phenomenex, Melbourne, Australia). PG samples were dissolved in an acetonitrile (ACN):chloroform, 80:20 (v/v) mixture, whilst OG and GMO samples were dissolved in an ACN:chloroform:methanol, 45:45:10 mixture prior to dilution in the starting mobile phase for HPLC. For irinotecan hydrochloride and irinotecan base a gradient elution method was used comprising A: acetonitrile, B: MilliQ water and C: 38% ACN/62% (50 mM Na_2HPO_4, 5 mM heptane sulfonic acid, adjusted to pH 3 with 85% orthophosphoric acid) over time course: 0–2 min, B = 30%, C = 70%; 2–7 min, 100% C; 7–10 min, A = 95%, B = 5%; 14–16 min, B = 30%, C = 70%, with flow rate = 1 mL/min and UV detection at 370 nm. For paclitaxel, the time course used was: 0–5 min, A = 70%, B = 30%; 5.5–9.5 min, A = 95%, B = 5%; 10–12 min, A = 70%, B = 30%; with flow rate = 1 mL/min and UV detection at 228 nm. Histidine was analysed using isocratic elution with 20 mM KH_2PO_4 buffer (pH 8.0) at 0.8 mL/min, and detection at 208 nm. Glucose was assayed using isocratic elution with 80%/20% ACN/MilliQ water, and with refractive index detection (Waters 2414 dRI; sampling rate: 2, sensitivity: 256, filter time: 1.0, temperature: 35 °C). Octreotide acetate was analysed at 0.8 mL/min with isocratic elution of 27%/73% ACN/0.1% (v/v) trifluoroacetic acid in MilliQ water and detected at 221 nm. The methods were validated by standard methods for precision and accuracy.

Table 1
Solubility of model drugs in neat liquid crystal-forming surfactants at 37 °C (values are mean ± S.D., $n = 3$)

	Solubility (mg/g)		
	Paclitaxel	Irinotecan HCl	Irinotecan base
Glyceryl monooleate	8.2 ± 0.4	2.12 ± 0.01	6.9 ± 0.2
Oleyl glycerate	8.4 ± 0.2	9.7 ± 0.7	35.7 ± 1.3
Phytanyl glycerate	5.0 ± 0.7	4.3 ± 0.4	64.6 ± 1.7

3. Results

3.1. Solubility of hydrophobic drugs in liquid crystal forming surfactants

The solubility of irinotecan base, irinotecan hydrochloride and paclitaxel in the various neat surfactants are provided in Table 1. The solubility of paclitaxel and irinotecan hydrochloride were generally low (<10 mg/g), whilst irinotecan base showed a moderate degree of solubility in the two glycerate-based surfactants, indicating the potential to load therapeutically relevant levels of this compound into these systems. In the context of these studies, the solubility values were all sufficient from an analytical perspective to permit the release studies to be performed, in order to compare drug release behaviour from the different lipid matrices.

3.2. Phase studies of glycerate surfactants

As illustrated in Fig. 2, glycerate-based surfactants were found to form a low viscosity L_2 phase at low water content, and a reverse hexagonal phase at higher water contents up to excess water at 37 °C. The phase boundaries determined by microscopy for the L_2 to H_{II} boundary, and Karl Fisher coulometry for the excess water boundary occurred at similar compositions for both glycerate surfactants, despite the very different molecular structure of the hydrophobic tails. In the case of OG, the transition from L_2 to H_{II} occurred at 7 ± 1% water, and the excess water boundary occurred at 29.9 ± 1.1%. For PG, the transition from L_2 to H_{II} similarly occurred at 7 ± 1% (w/w) water, and the excess water boundary occurred at 39.0 ± 2.7% (w/w) water.

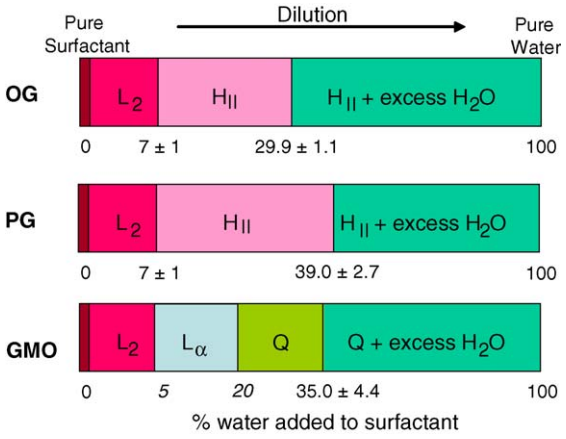

Fig. 2. One dimensional partial phase diagrams for OG, PG and GMO at 37 °C showing water content at phase boundaries for reverse micellar (L_2), lamellar (L_α), reverse hexagonal (H_{II}) and cubic phase (Q). Data in italics are derived from the published phase diagram as described in the text.

By way of comparison, GMO undergoes an L_2 to lamellar (L_α) and L_α to Q transitions with increasing water at approximately 5 and 20% (w/w) water, respectively (Clogston et al., 2000). The excess water boundary (Q to Q + excess H_2O) was determined to be at 34.9 ± 4.4% (w/w) water, which agrees well with the reported phase behaviour for Myverol.

Addition of the fatty alcohol to the surfactants induced a change from H_{II} to L_2 at the phase boundary with excess water for both glycerate surfactants. Using Karl Fisher coulometry, the water content of samples with increasing ratios of oleyl alcohol:oleyl glycerate and phytanol:phytanyl glycerate when equilibrated with excess water were determined, and are plotted in Fig. 3. The water content in the phase at equilibrium remains relatively unchanged at very low fatty alcohol levels and the H_{II} phase structure is maintained. As the alcohol content is increased closer to a critical value at which L_2 phase is formed, the water content increases dramatically, presumably through swelling of the matrix in preparation for the transition to reverse micelles, and the water content drops substantially on addition of further alcohol to the L_2 phase.

3.3. Release of hydrophobic drugs from pre-formed LC phases

The release of drug from the liquid crystalline matrices formed from OG, PG and GMO was assessed using three hydrophobic drugs, paclitaxel, irinotecan HCl and irinotecan base. Fig. 4A illustrates the difference in release rate of paclitaxel and irinotecan hydrochloride from the OG matrix compared to GMO. Clearly, the release rate of paclitaxel was more rapid from the GMO matrix, despite the solubility of paclitaxel being essentially identical, eliminating partition effects as reason for the difference under non-sink conditions. Similarly, the GMO matrix also provided faster release of irinotecan hydrochloride, however its lower solubility in GMO may have contributed to this difference. When the paclitaxel data is plotted as percent released versus square root of time, the data show a linear relationship, particularly at early times, indicating that diffusion is the dominant release mode. The slope of the linear fit for GMO was nearly 2.5 times greater than OG (0.658 and 0.277 $h^{-1/2}$, respectively), indicating much faster diffusion of paclitaxel through the cubic phase matrix compared to the reverse hexagonal phase.

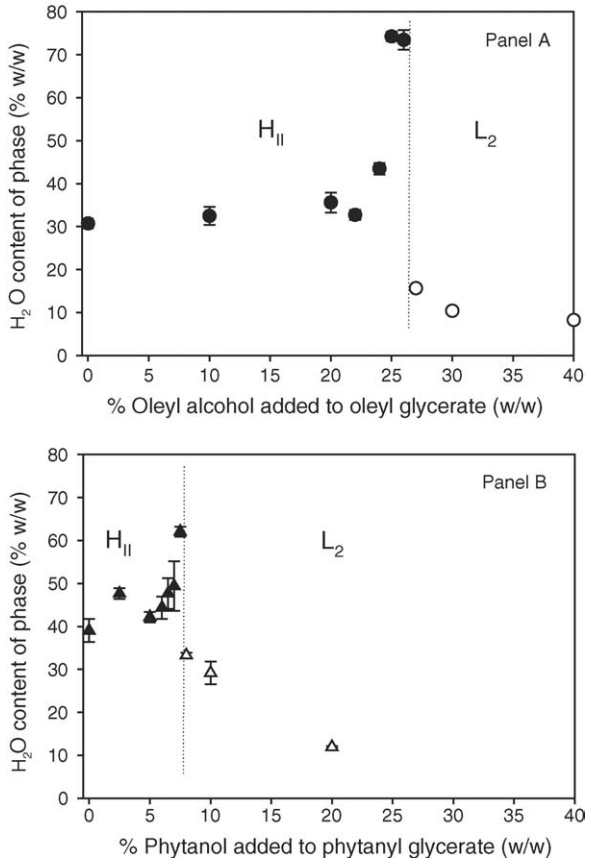

Fig. 3. The effect of fatty alcohols on the phase behaviour of OG (Panel A) and PG (Panel B) liquid crystalline matrices. The addition of increasing amounts of fatty alcohols at 37 °C in both cases led to conversion of the H_{II} phase (closed symbols) to L_2 phase (open symbols), accompanied by significant changes in the composition of the phase boundary with excess water. Values are mean ± S.D. (n = 3).

A similar comparison of the rate of drug release from PG compared to OG is provided in Fig. 5. In this case, the plot of percent released against square root of time indicates that the release of either irinotecan base and irinotecan hydrochloride from OG H_{II} phase provide essentially the same release rate constant, as the slopes are very similar. However, the release of irinotecan base from PG H_{II} phase is significantly slower than from OG H_{II} phase (slope = 0.658 and 0.277 $h^{-1/2}$ for release of irinotecan base from OG and PG H_{II} phase, respectively). This is a further indication that not only is the type of mesophase important, but also the material from which the liquid crystal phase is prepared.

3.4. Release of hydrophilic drugs from pre-formed LC phases

Glucose, histidine and octreotide acetate, were used as model hydrophilic drugs with varying physicochemical properties, i.e. small uncharged hydrophilic drug, small charged hydrophilic

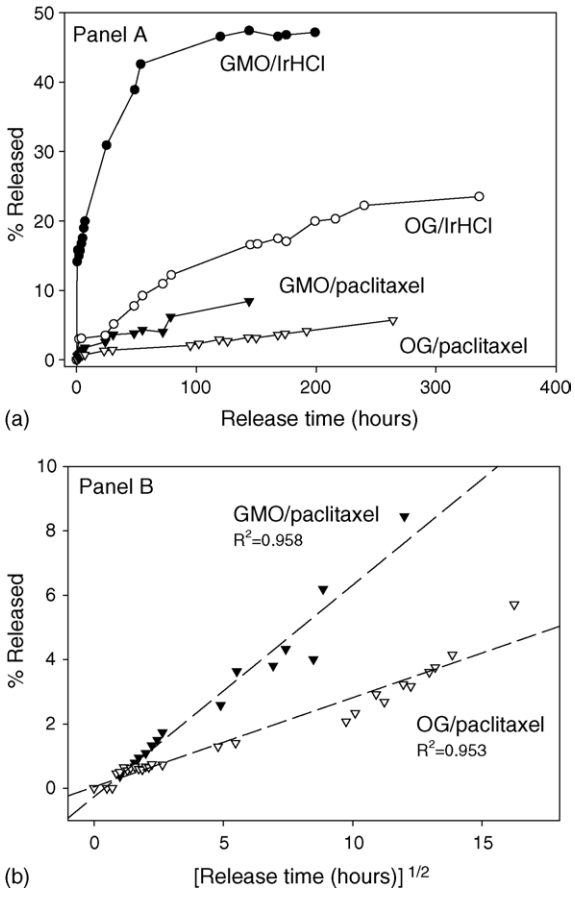

Fig. 4. The difference in release behaviour from reverse hexagonal phase (OG data) and cubic phase (GMO data) is highlighted, illustrating the more rapid release from GMO cubic phase. Panel A illustrates the raw release data obtained for irinotecan hydrochloride (circles, IrHCl) and paclitaxel (inverted triangles) from the GMO (closed symbols) and OG (open symbols) pre-formed liquid crystalline matrices. The lines are intended as a visual guide only. The paclitaxel data is re-plotted against the square root of time in Panel B to illustrate the dependence of drug release on diffusion. The dashed lines in Panel B are a least squares linear fit for the data in each case.

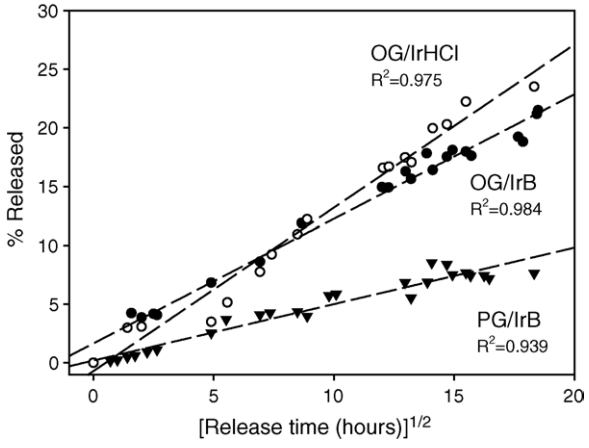

Fig. 5. The release of irinotecan base (IrB) from the H_{II} phase formed from OG (closed circles) is more rapid than from PG (closed triangles). Irinotecan base and hydrochloride (IrHCl, open circles) are released at very similar rates from the OG-based H_{II} matrix. With release rate plotted against square root of time, the release rates are shown to be strongly diffusion dependent. The dashed lines are a least squares linear fit for the data in each case, with the correlation coefficient included for each drug/surfactant combination.

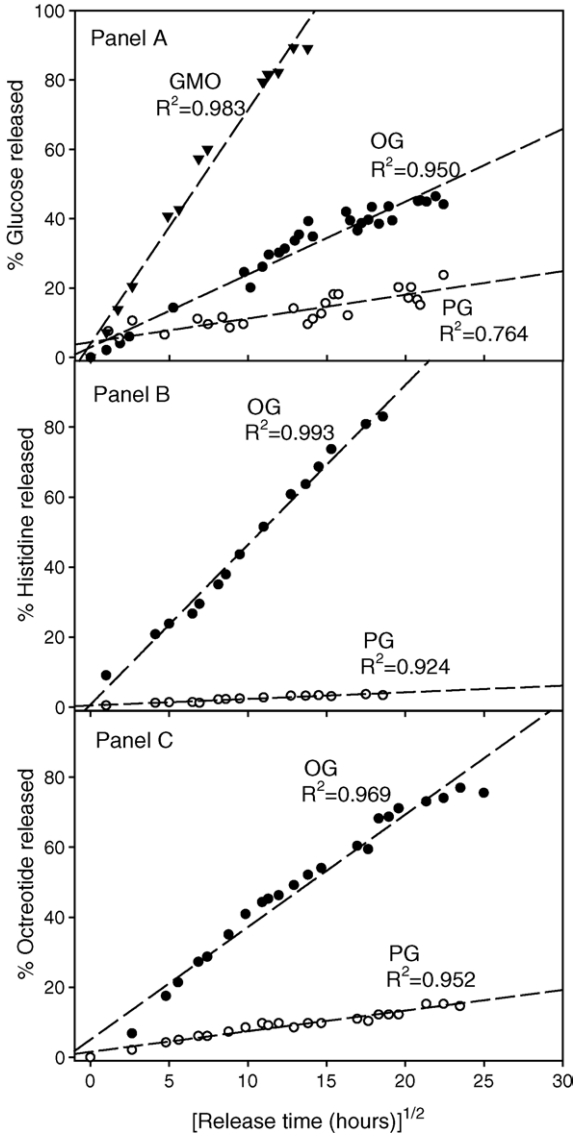

Fig. 6. The release of hydrophilic model drugs from the pre-formed liquid crystalline matrices prepared from GMO (inverted triangles), OG (filled circles) and PG (open circles) at 37 °C is shown. Panel A illustrates the release of glucose into PBS at pH 7.4; Panels B and C illustrate the release of histidine and octreotide into acetate buffer at pH 4.0. The dashed lines are a least squares linear fit for the data in each case, with the correlation coefficient included for each drug/surfactant combination.

drug, and larger hydrophilic drug, representative of a peptide, respectively. The release of each model drug from OG- and PG-based H_{II} phases is presented in Fig. 6A–C, respectively. Fig. 6A also illustrates glucose release from the cubic phase formed by GMO for comparison to the results obtained for paclitaxel. Again, these plots are percent released versus square root of time, and in each case there is a clear fit with diffusion controlled release. The issues of sink condition in this case are avoided as the concentration of model drug in solution at 100% release is very small compared to the solubility of the model drug in the release medium.

Table 2 summarises the results obtained from these profiles in the form of slope for each drug/surfactant combination illus-

Table 2
Slope of release profiles for hydrophilic drugs in liquid crystalline matrices formed from oleyl glycerate (OG), phytanyl glycerate (PG) and glyceryl monooleate (GMO) presented in Fig. 6

	Slope of % released vs. release time$^{1/2}$		
	OG	PG	GMO
Glucose	2.10	0.68	6.75
Histidine	4.56	0.19	nd
Octreotide	3.21	0.59	nd

Slope is proportional to apparent diffusion coefficient of the drug in the matrix; nd: not determined.

trated in Fig. 6. In all cases the release from the OG H_{II} phase was faster than from the PG H_{II} phase. In the case of histidine, release from the PG H_{II} matrix was over an order of magnitude slower than from the OG H_{II} phase. Release of glucose was three times faster from the GMO cubic phase than from the glycerate-based matrices, as was observed for the hydrophobic drugs. The linearity of the plots (R^2) was generally >0.9 indicating a strong dependence on diffusion for release of the hydrophilic drugs, in agreement with previous work on similar systems (Ericsson et al., 1991). The release of octreotide over approximately one month in duration further provides evidence of the potential of these simple systems as new therapeutic drug delivery alternatives.

3.5. Release of hydrophilic drugs from precursor systems

The liquid crystalline matrices, whilst providing promising release characteristics, are very viscous and improbable candidates for a clinically useful dose form. However, the phase behaviour illustrated in Fig. 2 for the glycerate-based surfactants shows that addition of up to 7% water permits the formulation of an aqueous solution of hydrophilic drug in the surfactant whilst retaining the low viscosity L_2 phase, and on exposure to excess aqueous solution, for example, in the subcutaneous environment, would provide in situ formation of the H_{II} phase containing the hydrophilic drug in the aqueous domains.

Fig. 7 illustrates the concept by observation of injection of such an L_2 formulation into water, when viewed through crossed polarisers. The first frame shows the injection of the isotropic L_2 phase comprising octreotide acetate solution and oleyl glycerate. Penetration of the aqueous medium into the L_2 phase on injection is analogous to moving to the right in the phase diagram in Fig. 2, and results in the formation of the viscous, birefringent H_{II} phase almost immediately. Drug is then released from this matrix. Fig. 8 illustrates the release of octreotide from the L_2 phase when prepared using OG and PG. Once again, the release from OG H_{II} phase was much faster than from the PG H_{II} phase. However, release from the injected precursor form of OG was more rapid than from the pre-formed matrix (Fig. 6C), likely due to the larger surface area from which the drug is released after dissolution of the capsule. Release from the PG precursor matrix was very similar to that from the pre-formed H_{II} matrix. From these studies it is not apparent why this should be the case, but virtually nothing is known about how these glycerate-based materials swell and the mechanisms of phase transitions, which may hold some clue as to the differences in behaviour. Nevertheless, a clear distinction between release rates from the two lipid matrices is again reflected in these data. The release of drug from the in situ formed matrices still showed strong diffusion dependence as before. Importantly, there does not appear to be a 'burst release' event often associated with injectable depot systems prepared using PLGA (Brodbeck et al., 1999).

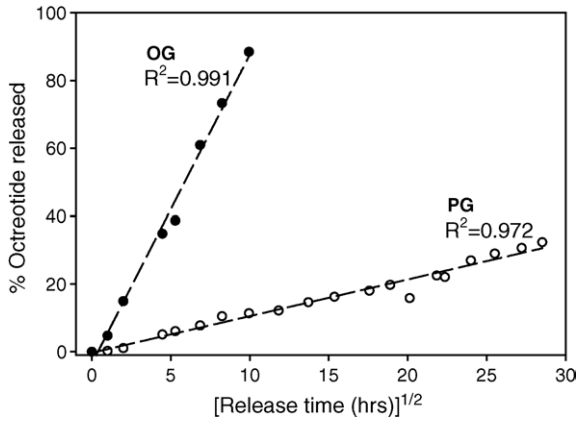

Fig. 8. Release of octreotide from OG and PG-based injectable precursor systems at 37 °C (closed and open circles, respectively) into pH 4 acetate buffer. The dashed lines are a least squares linear fit for the data in each case, with the correlation coefficient included for each drug/surfactant combination.

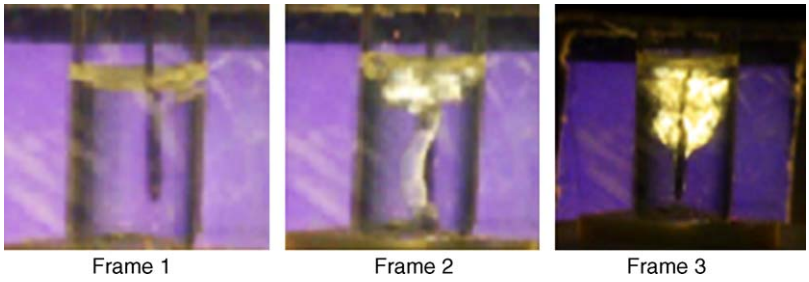

Fig. 7. Injection of an OG-based precursor containing octreotide into pH 4.0 acetate buffer viewed through crossed polarizing filters. In Frame 1, the isotropic (non-birefringent) L_2 liquid precursor rises to the surface of the isotropic buffer on injection. Soon thereafter in Frame 2, sufficient water is absorbed to begin the in situ formation of the bright, birefringent H_{II} phase from the non-birefringent L_2 phase. The bright mass of material in Frame 3 is the reverse hexagonal phase matrix from which sustained release of octreotide then occurs. The photographs were taken at approximately 5 s intervals.

4. Discussion

The liquid crystalline systems studied here provide an opportunity for a very simple versatile platform delivery system for hydrophilic and hydrophobic drugs. In principle, hydrophobic drugs could be dissolved in the lipid and injected directly to form the sustained release depot in situ. The ability to formulate hydrophilic drugs into a sustained release matrix without the drawbacks associated with the PLGA systems is attractive; the L_2 phase precursor systems described in this report could be adapted to existing immediate release parenteral formulations. For example, a lyophilised peptide could be reconstituted in 7 parts aqueous phase and 93 parts surfactant, mixed to form the homogeneous, low viscosity L_2 phase, and injected to form a sustained release depot in situ.

A similar approach for the formulation of a low viscosity precursor has been investigated for the GMO cubic phase matrix. Chang and Bodmeier investigated systems containing chlorpheniramine maleate and propranolol, in which drug or a solvent were added to the swollen cubic phase to form a lower viscosity lamellar phase that would facilitate injection (Chang and Bodmeier, 1998). On injection, initial release of the drug or solvent from the matrix transformed the matrix to cubic phase providing a sustained release effect. Combination systems of GMO/PLGA and solvent have also been proposed (Johansson et al., 2001). GMO also forms an L_2 phase at low water contents, below that required to form the lamellar phase, however Chang and Bodmeier chose the above approaches for formulating the injectable systems due to concerns about the possibility of irritation due to uptake of water from surrounding tissues if partially swollen systems were used. In the context of the current study, this may be a future limitation of the technology, but would need to be evaluated in vivo.

One limitation of the precursor system described in this work is the relatively rapid release, at least in the case of the OG precursor illustrated in Fig. 8. However, there was a dramatic difference between the OG and PG systems in this configuration; whilst the OG matrix was essentially exhausted of drug within 4 days, drug supply from the PG matrix would not be exhausted until approximately 10,000 h, or over one year if the PG system behaved essentially linearly at much longer times than those followed in these experiments. In practice, the rate of drug release at long times would be so slow that it would be unable to provide a therapeutically relevant supply of drug to the body and in vivo conditions are likely to result in breakdown and/or dispersion of the matrix over time leading to more rapid drug release. The increasing number of materials being discovered that form Q and H_{II} phases in excess water will lead to a better ability to manipulate the release rates.

The release rate is controlled by a number of factors related to both the drug and the matrix itself. The diffusion coefficient of the drug is dependent on its hydrodynamic radius, i.e. largely on its molecular weight. Interaction of a hydrophilic drug with the lipid matrix through e.g. hydrogen bonding or ion-pairing may also alter the release rates by reducing the diffusion coefficient. If drug is transported primarily through the aqueous domains of the matrix, then the access of the aqueous domains to the external aqueous solution is also likely to be an important factor in drug escape from the matrix. More rapid release of all of the model drugs from cubic phase compared to H_{II} phase can be attributed to the differences between the geometry of the aqueous domains. The cubic phase matrix consists of two aqueous water channels that penetrate throughout the matrix (Hyde et al., 1984) and provide the principal route of drug release for both lipophilic and hydrophilic drugs. These aqueous domains are likely open to the external aqueous phase. In the case of the H_{II} phase, the aqueous compartments are closed extended micellar columnar structures (Laughlin, 1994). Hydrophilic drug that is in these compartments would require random perturbation/dynamic destruction-reconstruction events of the reverse micelles to provide for drug release. Hence, it is understandable that the release of drug from GMO cubic phase is more rapid than from the glycerate hexagonal phases.

PLGA releases drug primarily by erosion of the biodegradable polymer matrix, whilst for the liquid crystalline systems presented in this paper, diffusion of drug from a passive matrix dominates the release behaviour. This has important therapeutic implications and opportunities, as the rate of drug release slows over time from the liquid crystalline matrix, whilst the polymer-based systems are usually tailored to provide a zero order constant release of drug. In some circumstances, the diffusion controlled release may be beneficial in providing a loading dose of drug initially, then a slower maintenance at longer times before tailing off below therapeutic levels. Dual or biphasic release kinetics of this kind has potential benefits in a clinical scenario where the two ‘release regimes’, i.e. a dual injection providing both therapeutic benefits of loading dose and longer term therapeutic maintenance. Often drugs will display a therapeutic toxicity profile that can be improved by incorporation of the drug into a sustained release oily vehicle (Larsen et al., 2002). It was shown that it is possible to develop in vitro—in vivo correlations for these types of systems.

The differences in release behaviour between the OG and PG matrices, which occurred for the release of all the model drugs used in these studies is less readily explained. Both materials form H_{II} phases at similar compositions (Fig. 2), thus differences in aqueous domain volume is not sufficient to explain these differences. There appears to be no explanation that can be drawn directly from these data. Future experiments using small angle X-ray scattering, fluorescence anisotropy and self-diffusion NMR are being planned to attempt to elucidate the reasons for these differences.

The release of drugs with a wide range of physicochemical properties from GMO cubic phase has been investigated and the matrices have been shown to be potentially useful delivery vehicles, yet no injectable depot products have progressed into clinical development. Whilst the reasons for this have not been provided, it is possibly due to the potential for GMO to be degraded to oleic acid by non-specific esterases, which in turn may form calcium oleate deposits in tissue. Calcium oleate has been previously reported to cause tissue necrosis (Appel and Jensen, 1994). In the case of the glycerate esters of this study, the product is the fatty alcohol. The susceptibility of the glyc-

erates to esterases, and the tolerability and local toxicity of the degradation products are at this stage unknown, and will need to be determined.

There is promising potential for glycerate surfactants to provide a controlled release matrix for administration other than as a parenteral depot injection. Recently, the utility of cubic phase for transdermal drug delivery has been explored, as has the use of cubic phase as an artificial vernix for premature babies (Feuer, 2003; Turchiello et al., 2003). The lack of fatty acids in the glycerates may improve their local tolerability profiles compared to GMO-based systems. The potential application of these materials in oral drug delivery is also being explored.

It is now well known that materials displaying phase behaviour similar to that exhibited by these surfactants in Fig. 2, can be dispersed into particle form and employed as a drug carrier analogous to liposomes, the so called Cubosomes™ and Hexosomes™. Their preparation is relatively straight forward, and because the phase prior to dispersion is stable in excess water, the particles do not dissolve or transform into other structures on dilution. Thus, it is anticipated that glycerate surfactants would form hexosomes at physiological temperature, and we plan to investigate this possibility further in forthcoming publications. Glycerate surfactants may overcome the issues associated with GMO cubosomes in terms of toxicity and lower temperature stability, as the lipid is not based on fatty acids, and in the case of PG has a melting point below 5 °C.

5. Conclusions

In this in vitro study, we have demonstrated the potential utility of a new class of mesophase-forming surfactants with glycerate headgroups, in providing a sustained release matrix. Changing the hydrophobe structure of the surfactants has been shown to alter the release characteristics in a consistent manner. The glycerate surfactants, which form reverse hexagonal phase in excess water, consistently displayed a slower release of incorporated hydrophilic and hydrophobic drugs than glyceryl monooleate, which is known to form cubic phase in excess water. The concept of preparing a drug-containing L_2 phase precursor that forms reverse hexagonal phase on exposure to excess aqueous solutions has also been demonstrated and sustained release observed on injection into excess solution, simulating a subcutaneous depot delivery scenario. These novel surfactants provide an alternative material to GMO from which to prepare liquid crystalline sustained release systems for drug delivery.

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References

Appel, L.K.E., Jensen, J.L.R.G.Z., 1994. An in vitro model to mimic in vivo subcutaneous monoolein degradation. Pharm. Res. 11, S-217.

Asherman, J., Ye, Q., Stevenson, M., Katre, N., 1998. DepoFoam™ Technology: an effective vehicle for controlled delivery of proteins and peptides. J. Control. Release 25, 503.

Barauskas, J., Landh, T., 2003. Phase behaviour of the phytantriol/water system. Langmuir 19, 9562–9565.

Boyd, B.J., Davey, G., Drummond, C.J., Hartley, P., Fong, C., Krodkiewska, I., Murphy, A., Tait, R., Warr, G., Wells, D., Whittaker, D.V., Ye, R., 2004. Surfactants and lyotropic phases formed therefrom. International patent WO 2004/022530, March 2004.

Boyd, B.J., Davey, G., Khoo, S.-M., Whittaker, D.V., 2005. Compositions and methods of delivery of biologically active agents. International patent WO2005021046, March 2005.

Brodbeck, K.J., DesNoyer, J.R., McHugh, A.J., 1999. Phase inversion dynamics of PLGA solutions related to drug delivery. Part II. The role of solution thermodynamics and bath-side mass transfer. J. Control. Release 62, 333–344.

Chang, C.-M., Bodmeier, R., 1998. Low viscosity monoglyceride-based drug delivery systems transforming into a highly viscous cubic phase. Int. J. Pharm. 173, 51–60.

Clogston, J., Rathman, J., Tomasko, D., Walker, H., Caffrey, M., 2000. Phase behaviour of a monoacylglycerol (Myverol 18–99 K)/water system. Chem. Phys. Lipids 107, 191–220.

Drummond, C.J., Fong, C., 1999. Surfactant self-assembly objects as novel drug delivery vehicles. Curr. Opin. Colloid Interface Sci. 4, 449–456.

Ericsson, B., Eriksson, P.O., Löfroth, J.E., Engström, S., 1991. Cubic phases as delivery systems for peptide drugs. In: ACS Symposium Series, pp. 251–265.

Feuer, J., 2003. Cubosome Technology will Benefit Premature Babies. Cincinnati Children's Hospital Medical Centre News Release, September 2003 (online at http://www.cincinnatichildrens.org/about/news/release/2003/9-cubosome-technology.htm).

Higuchi, W.I., 1967. Diffusional models useful in biopharmaceutics. J. Pharm. Sci. 56, 315–324.

Hyde, S.T., Andersson, S., Ericsson, B., Larsson, K., 1984. A cubic structure consisting of a lipid bilayer forming an infinite periodic minimum surface of the gyroid type in the glyceryl monooleate-water system. Z. Kristallogr. 168, 213–219.

Johansson, A.K., Linse, P., Piculell, L., Engström, S., 2001. Phase behavior of the quaternary poly(DL-lactide-co-glycolide)/monoolein/1-methyl-2-pyrrolidinone/water system: an experimental and theoretical study. J. Phys. Chem. B 105, 12157–12164.

Larsen, D.B., Joergensen, S., Olsen, N.V., Hansen, S.H., Larsen, C., 2002. In vivo release of bupivacaine from subcutaneously administered oily solution. Comparison with in vitro release. J. Control. Release 81, 145–154.

Laughlin, R.G., 1994. The Aqueous Phase Behaviour of Surfactants. Academic Press, London.

Lawrence, M.J., 1994. Surfactant systems: their use in drug delivery. Chem. Soc. Rev. 23, 417–423.

Minamikawa, H., Hato, M., 1997. Phase behaviour of synthetic phytanyl-chained glycolipid/water systems. Langmuir 13, 2564–2571.

Mueller-Goymann, C.C., Frank, S.G., 1986. Interaction of lidocaine and lidocaine-HCl with the liquid crystal structure of topical preparations. Int. J. Pharm. 29, 147–159.

Okumu, F.W., Dao, L.N., Fielder, P.J., Dybdal, N., Brooks, D., Sane, S., Cleland, J.L., 2002. Sustained delivery of human growth hormone from a novel gel system: SABER™. Biomaterials 23, 4353–4358.

Qui, H., Caffrey, M., 1998. Lyotropic and thermotropic phase behaviour of hydrated monoacylglycerols: structure characterisation of monovaccenin. J. Phys. Chem. B 102, 4819–4829.

Rosevear, F.B., 1968. Liquid crystals: the mesomorphic phases of surfactant compositions. J. Soc. Cosmet. Chem. 19, 581–594.

Shah, J.C., Sadhale, Y., Chilukuri, D.M., 2001. Cubic phase gels as drug delivery systems. Adv. Drug Deliv. Rev. 47, 229–250.

Turchiello, R.F., Vena, F.C.B., Maillard, P., Souza, C.S., Bentley, M., Tedesco, A.C., 2003. Cubic phase gel as a drug delivery system for topical application of 5-ALA, its ester derivatives and m-THPC in photodynamic therapy (PDT). J. Photochem. Photobiol. B Biol. 70, 1–6.