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Hexosomes formed from glycerate surfactants—Formulation as a colloidal carrier for irinotecan

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Abstract

A new class of amphiphiles with a glycerate headgroup, recently shown to form reverse hexagonal phase in excess water, have been dispersed to form Hexosome dispersions comprising sub-200 nm particles retaining the internal nanostructure of the parent H_{II} phase. The application of these novel materials to the development of a new injectable formulation of irinotecan was investigated. The formulation of irinotecan with a small percentage of oleic acid in oleyl glycerate permitted a clinically relevant dose of irinotecan to be dissolved in the glycerate surfactant and dispersed in aqueous medium to form an injectable particle-based dose form of irinotecan. Importantly, incorporation of irinotecan into Hexosomes at neutral pH did not result in conversion from the active lactone to the inactive carboxylate form on storage, and is hence a promising alternative to the current low pH formulation of irinotecan required to inhibit this conversion. Although release of irinotecan from the Hexosomes was shown to be virtually instantaneous from the Hexosomes on substantial dilution, the retention of the drug in lactone form at neutral pH demonstrates a potential application of these novel nanostructured particles in injectable drug delivery.

Keywords: Nanostructured lipid carriers; Liquid crystalline phases; Irinotecan; Formulation; In vitro release

1. Introduction

Lipid-based liquid crystalline materials, formed by the swelling of certain polar lipids when exposed to aqueous environments, have received much recent attention in the drug delivery field for their ability to sustain the release of a wide range of molecules (Drummond and Fong, 1999). The liquid crystalline matrices possess distinct lipidic and aqueous domains, and may exhibit a number of well-defined geometric arrangements depending on the chemical structure of the lipid, the aqueous content of the system, the presence of other additives, and solution conditions such as pH, temperature, pressure. Most often this arrangement consists of lamellar bilayer structures, but for a relatively small subset of lipids, the exhibited phase structures may include the viscous reverse hexagonal phase (H_{II}) or bicontinuous cubic phase (Q) (Laughlin, 1994; Hyde et al., 1997).

An even smaller subset of these materials swell with only a finite proportion of aqueous solution, and at greater water content retains the liquid crystalline structure with the coexisting aqueous solution. Until recently, there have been only a few materials known to exhibit this kind of phase behaviour; glyceryl monooleate (GMO), and other closely related unsaturated monoglycerides are the best known molecules of this class (Briggs and Caffrey, 1994a, 1994b; Qui and Caffrey, 1998, 1999). Materials with a phytanyl chain, such as phytantriol (Barauskas and Landh, 2003), and a glycolipid consisting of xylitol with a phytanyl tail (beta-XP) (Hato and Minamikawa, 1996) also display this phase behaviour on increasing water content. A new class of lipids that display finite swelling of H_{II} and Q phases, based on glycerate and urea headgroups has recently been disclosed (Boyd et al., 2004). Of these materials, phytanyl glycerate (PG) and oleyl glycerate (OG), illustrated in Fig. 1, were found to form reverse hexagonal phase in excess water at physiological temperature.

The finite swelling phenomenon is important for the potential use of lipid-based liquid crystals in drug delivery, as it provides

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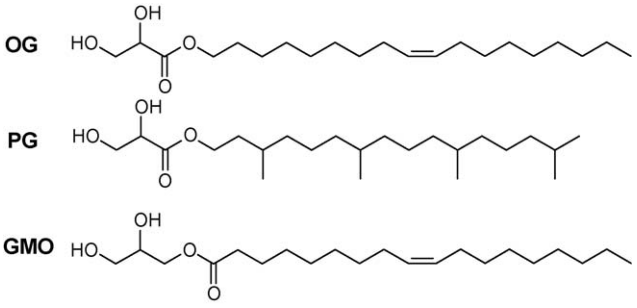

Fig. 1. Structures of oleyl glycerate (OG), phytanyl glycerate (PG) and glyceryl monooleate (GMO).

an opportunity to form a lipidic matrix which, on exposure to excess bodily fluids following administration, forms a persistent matrix for both lipophilic and hydrophilic drugs from which drug can be released (Chang and Bodmeier, 1998; Drummond and Fong, 1999; Shah et al., 2001). The hydrophobic domains can also provide a medium for enhancing the solubility of poorly water-soluble drugs. We have recently described the release of both hydrophobic and hydrophilic drugs from the H_{II} phase formed by the PG and OG polar lipids in Fig. 1, and the release of drug from these systems has been demonstrated to be solely through diffusion (Boyd et al., 2006).

A second important consequence of the finite swelling phenomenon is that the bulk lyotropic liquid crystals can be dispersed into sub-micron particles in excess aqueous solution, and still retain their internal structure (Larsson, 1989). The dispersion of the H_{II} and Q phases results in particles termed Cubosomes and Hexosomes, respectively. Cubosomes and Hexosomes have been almost exclusively prepared using fatty acid based amphiphiles such as GMO (Gustafsson et al., 1997; Spicer and Hayden, 2001); until the recent report of submicron dispersions of hexosomes prepared with glycerate surfactants (Fong et al., 2005), the only non-fatty acid based liquid crystalline particle system was that described by Abraham, et al. for beta-XP (Abraham et al., 2004).

There is a developing interest in the potential of colloidal delivery systems to provide a slow release matrix to replace the need for long, inconvenient and expensive infusion regimes, and utilizing the particles as a means to protect otherwise labile drugs from degradation (Chung et al., 2002). Cubosomes and Hexosomes have been postulated to potentially achieve these aims, but their full potential in this field has yet to be realised.

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin) is a water-soluble camptothecin, that shows excellent activity against colorectal cancer. Following administration the lactone ring that is present in the irinotecan structure rapidly converts to the inactive carboxylate isomer (Fig. 2), and undergoes concurrent cleavage of the bipiperidino-side chain to the highly cytotoxic SN-38 (7-ethyl-10-hydroxycamptothecin) (Mathijssen et al., 2001). Conversion to the carboxylate forms is minimal at pH 3.5. Consequently the commercial form of irinotecan, Camptosar® is formulated at pH 3.5. The major side effects of irinotecan therapy, primarily debilitating diarrhoea and bladder toxicity, are believed to be due to the excretion of SN-38 (Rivory, 1996), and consequently a reduction in overall dose but maintaining the effectiveness of this drug would be favourable. Our interest in irinotecan and dispersed liquid crystalline particles have converged in this study into the potential for these colloidal carriers to maintain irinotecan in its lactone form at neutral pH. The potential for the dispersed liquid crystalline particles to provide a sustained release dose form is also of interest in the context of the general utility of these particles in drug delivery.

Consequently, the aims of these studies are two fold. Firstly, we aim to demonstrate the preparation of liquid crystalline particles using glycerate-based polar lipids. Second, we describe the formulation and colloidal stability of glycerate-based liquid crystalline particles containing irinotecan. The stability of the dispersed lipids, particle size and irinotecan disposition were studied over time. Drug release from the non-dispersed and dispersed formulations of irinotecan was also investigated.

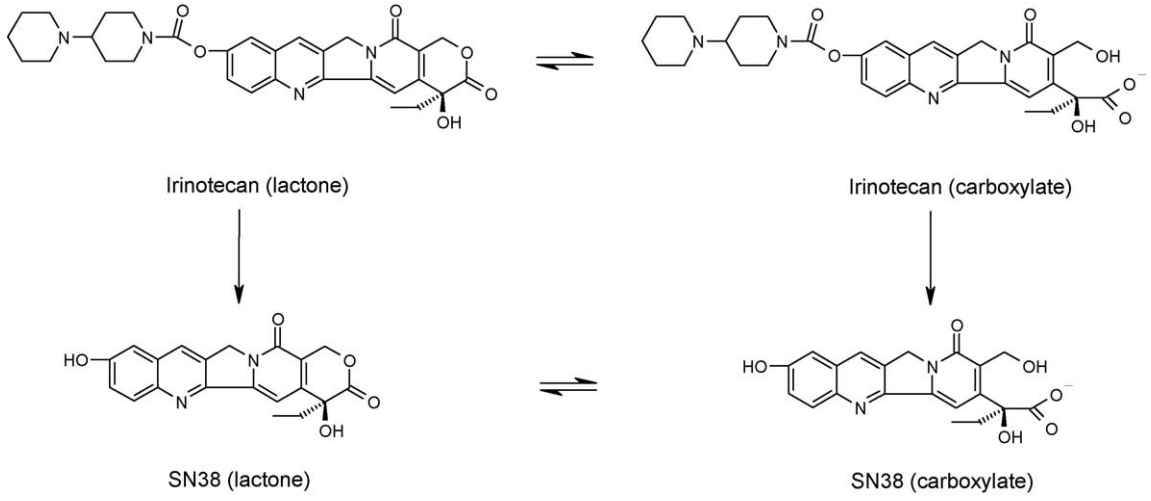

Fig. 2. Structures of irinotecan and SN-38 in the lactone and carboxylate forms.

2. Materials and methods

2.1. Materials

Oleyl glycerate and phytanyl glycerate were manufactured at CSIRO-Molecular Science, Australia as described previously (Boyd et al., 2004) and were 99% and 96% pure by reverse phase HPLC, respectively. Myverol 18-99 (GMO) was obtained from Quest International. Myverol 18-99 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 considered to be a good model for GMO. Irinotecan base was obtained from Dabur, India and was used as received. Oleic acid (99%) and sorbitol were sourced from Sigma (St. Louis, MO). Pluronic F127 (Poloxamer 407) was injectable grade material sourced from BASF (Ludwigshafen, Germany).

2.2. Solubility

Due to the limited quantities of the oleyl glycerate and phytanyl glycerate, and the cytotoxicity of irinotecan, solubility studies of irinotecan were conducted in small 250 μL amber vials. Samples were prepared by weight. Using a microspatula, excess irinotecan was added to approximately 200 mg of lipid in triplicate. The mixtures were manually mixed and stored at 37 °C before centrifugation and sampling. An accurately weighed mass of the solution (∼20 mg) was removed and dissolved in 1 mL of an acetonitrile:chloroform:methanol mixture (45:45:10%, v/v/v). Irinotecan content was determined by HPLC as described previously (Boyd et al., 2004).

2.3. Bulk phase behaviour and water content

Crossed polarised optical microscopy was utilised to determine phase identity by observation of birefringence when in contact with excess aqueous solution (Rosevear, 1954, 1968). An Axiovert inverted microscope (Zeiss, Melbourne, Australia) and heating stage (Mettler Toledo, Melbourne, Australia) were used for this purpose, and conducted as described previously (Boyd et al., 2006). Water content in equilibrated liquid crystal samples was determined by Karl Fisher coulometry, also described previously (Boyd et al., 2006).

2.4. Production and characterization of hexosomes

Dispersions were prepared in a similar manner to that described by Gustafsson et al. (1997). Briefly, the lipid components and Poloxamer 407, and drug where present, were heated to 60 °C to provide a clear solution. The aqueous medium (25 mL) was also heated to 60 °C. A coarse dispersion was produced by injecting the lipidic solution into the aqueous medium whilst homogenizing with an UltraTurrax T25 tissue homogenizer (IKA, Staufen, Germany) at 11,000 rpm using an S25N-18G dispersing element for 90 s. The dispersion was immediately transferred to a thermostatted Avestin C5 homogenizer (Avestin, Ottawa, Canada) at 60 °C, and the coarse dispersion refined through 10 passes at 10,000 psi. The final dispersion was collected into a glass vial containing a magnetic stirrer bar and cooled to room temperature under stirring for 30 min before particle size analysis.

Particle size and zeta potential were measured using a Malvern Zetasizer 3000 (Malvern Instruments, Malvern, UK) and dispersions were diluted 1 in 100 with water prior to measurement for optimal measurement sensitivity. Analysis used the Contin algorithm and particle size values reported are Z average values for particle diameter. SAXS and cryo-TEM were described in the previous publication on dispersed glycerate surfactant systems (Fong et al., 2005). Osmolality was measured using an Osmomat 010 cryoscopic osmometer by Gonotec GmbH (Germany).

2.5. Drug release

Drug release from the bulk liquid crystalline phase was as described previously (Boyd et al., 2006). Briefly, the lipid(s) and irinotecan were combined and equilibrated with a slight excess of Milli-Q water (40%, w/w) at 37 °C for 100 h on a roller mixer. The bulk phase was loaded into a microbeaker (approximately 500 mg) and immersed in a thermostatted beaker at 37 °C containing the release medium (500 mL of Milli-Q 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. Irinotecan was analysed by HPLC as previously described (Boyd et al., 2006).

Drug release from Hexosomes was conducted by pressure ultrafiltration using a Millipore YM10 membrane in an Amicon 8050 magnetically stirred pressure ultrafiltration cell (Millipore, Australia). This method has been previously described in detail for drug release from Cubosomes (Boyd, 2003). Briefly, the Hexosome dispersion was diluted 1 in 100 in water to stimulate drug release from the Hexosomes, and at required time points the cell was pressurized to force free drug solution through the membrane for collection and analysis. In each case 900 μL was discarded prior to collection of the 100 μL sample as this amount of irinotecan far exceeded that required to saturate the membrane in previous experiments.

2.6. Analytical methods

Irinotecan was quantified by HPLC based on the method reported by Rivory and Robert (1994) using an Altima C8 5 μm, 250 mm × 4.6 mm column (Alltech, Melbourne, Australia). Irinotecan was eluted with mobile phase comprising 38% ACN/62% (50 mM Na_2HPO_4, 5 mM heptane sulfonic acid, adjusted to pH 6.4 with 85% orthophosphoric acid) in isocratic mode on a Waters Alliance HPLC system with flow rate of 1 mL/min and UV detection at 370 nm. The method was validated by standard methods for precision and accuracy. In order to trap the lactone and carboxylate forms of irinotecan, the samples were immediately diluted in HPLC mobile phase to prevent conversion between the two forms as described previ-

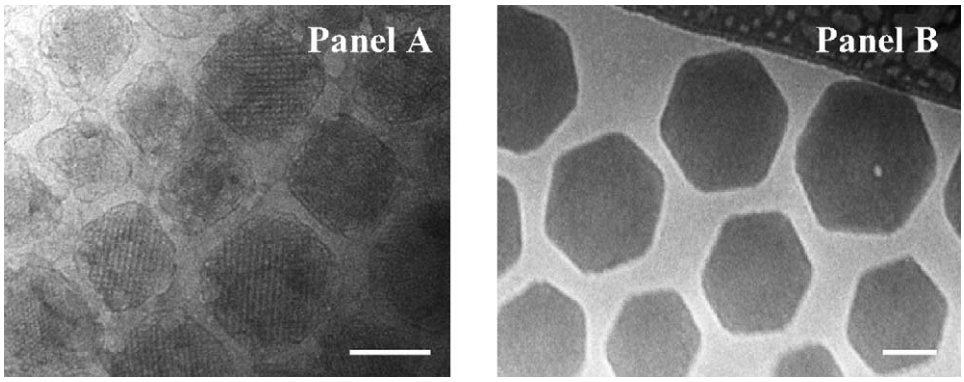

Fig. 3. Cryo-TEM image of a cubosome dispersion (Panel A) made from Myverol 18–99 K, and a Hexosome dispersion prepared from phytanyl glycerate (Panel B) in water. Both dispersions contain 10% w/w of the lipid (GMO or PG) and 0.1% w/w Poloxamer 407. The white bar represents 100 nm.

Table 1
Particle size of GMO-based Cubosomes and glycerate-based Hexosomes by PCS (Z-ave) and visual measurement from cryo-TEM images

	Particle size by PCS ($n = 3$ separate samples)		From cryo-TEM image (nm)
	Diameter (nm)	PI	Diameter
GMO Cubosomes	141.5 ± 0.8	0.124 ± 0.012	173 ± 28 ($n = 37$)
OG Hexosomes	178.6 ± 1.2	0.038 ± 0.025	–
PG Hexosomes	214.3 ± 1.7	0.083 ± 0.014	112 ± 29 ($n = 22$)

PI: polydispersity index.

ously by Rivory. Oleyl alcohol was quantified using a Zorbax Extend C18 column, 150 mm × 4.6 mm, 5 μm (Alltech Associates, Melbourne, Australia) and eluted with a mobile phase comprising 90% acetonitrile, 10% Milli-Q water with UV detection at 200 nm.

3. Results

3.1. Preparation of placebo dispersions

Placebo Hexosomes (without irinotecan) were prepared to demonstrate that it was possible to make dispersions of the novel glycerate lipids using the hot-melt method with Poloxamer 407 as steric stabilizer, as used previously to prepare GMO-based Cubosomes (Gustafsson et al., 1997). The data here build on the recent report of the preparation of dispersions of glycerate surfactants (Fong et al., 2005) and form the baseline data with which to compare the later drug loaded particles. Their properties have been described recently, together with detail on the SAXS and cryo-TEM results (Fong et al., 2005).

Cryo-TEM images, such as those in Fig. 3 were used for comparison of particle size and visual observation of particle morphology. Panel A illustrates Cubosomes prepared from GMO having a square profile, surrounded by small vesicular structures. This is consistent with previous findings for Cubosomes observed with cryo-TEM (Spicer, 2005). Panel B shows a cryo-TEM image of Hexosomes prepared from phytanyl glycerate. It demonstrates the remarkably clear hexagonal profile of the particles. The apparent absence of other species, such as the vesicular structures formed with GMO Cubosomes is not surprising in consideration of the fact that the glycerate surfactants do not exhibit a lamellar phase at any water content in the binary surfactant water phase behaviour.

Table 1 contains the particle size of placebo dispersions measured by PCS and cryo-TEM. The sizes of the particles in the cryo-TEM images are in apparent agreement with the PCS results. The methodology used to prepare GMO-based Cubosomes provides glycerate surfactant dispersions with similar particle size to Cubosomes.

3.2. Solubility and phase behaviour of irinotecan in glycerate surfactants prior to formation of Hexosomes

In order for the irinotecan-containing particulate dispersion to be useful, a meaningful benchmark dose of drug was set at

Table 2
Solubility of irinotecan in glycerate surfactant/oleic acid (% w/w) mixtures and lyotropic phase behaviour at 37 °C

Surfactant/oleic acid mixture	Solubility of irinotecan (mg/g)	Lyotropic phase formed in excess water
Oleyl glycerate	12.0 ± 1.3[a]	H_{II}
Phytanyl glycerate	26.7[b]	H_{II}
Oleic acid	226.8 ± 7.3[a]	L_2
Oleyl glycerate + 5% oleic acid	47.4[c]	H_{II}
Oleyl glycerate + 6% oleic acid	>50[d]	H_{II}
Phytanyl glycerate + 10% oleic acid	>50[d]	L_2
Myverol 18-99 (GMO)	6.9 ± 0.2[a]	Q

Q: cubic phase; H_{II}: reverse hexagonal phase; L_2: reverse micellar/fluid isotropic phase.

[a] Mean ± S.D., $n = 3$.

[b] Mean of duplicate determination.

[c] Single determination.

[d] Single observation of complete dissolution of drug in surfactant/oleic acid mixture.

Table 3
Optimization of Poloxamer 407 content for irinotecan-loaded oleyl glycerate Hexosome dispersions stored at 25 °C for 7 days

Poloxamer (% w/w)	Particle size (nm)		Polydispersity index	
	Day 0	Day 7	Day 0	Day 7
0.5	207.7 ± 0.8	265.6 ± 1.1	0.035 ± 0.017	0.089 ± 0.028
0.75	180.8 ± 1.2	244.4 ± 1.5	0.046 ± 0.001	0.162 ± 0.007
1.0	156.3 ± 0.4	241.8 ± 7.7	0.060 ± 0.010	0.210 ± 0.010

50 mg of irinotecan per 10 mL of 10% lipid dispersion. This dictated that a minimum solubility of irinotecan in lipid of 50 mg/g at 37 °C was required. The solubility of irinotecan was measured in both lipids and are listed in Table 2. The solubility of irinotecan in the neat lipids failed to meet this criteria, with solubility of approximately 12 and 27 mg/g irinotecan in OG and PG, respectively. By way of comparison, the solubility of irinotecan in Myverol 18-99 was determined to be 6.9 ± 0.2 mg/g, and is of similar magnitude to that in oleyl glycerate.

Basic lipophilic drugs often have a high solubility in oleic acid, due to the potential formation of lipophilic ion pairs; the solubility of irinotecan in oleic acid was measured to be 226.8 ± 7.3 mg/g (mean ± S.D., $n = 3$). Consequently, attempts to boost the solubility of irinotecan in the surfactants via the formation of lipophilic ion pairs with oleic acid added to the lipids provided the necessary drug loading. The addition of oleic acid at 6% (w/w) of total oleyl glycerate + oleic acid provided the necessary drug solubility, and optical microscopy indicated that the mixture retained its ability to form reverse hexagonal phase (H_{II}) in excess water. Although the solubility of irinotecan was greater in phytanyl glycerate than oleyl glycerate, the addition of oleic acid to phytanyl glycerate resulted in the formation of the low viscosity L2 phase, indicating that formation of Hexosomes on dispersion would be unlikely, and phytanyl glycerate was not pursued further in these studies.

3.3. Irinotecan Hexosome dispersions—optimization of Poloxamer 407 content and osmolality

Three levels of Poloxamer 407 (at 1, 0.75 and 0.5%, w/w of the dispersions), were tested to determine the minimum level of Poloxamer required to provide a stable dispersion for 1 week. Table 3 lists the particle size for each Hexosome dispersion at the time of manufacture and after storage at 25 °C for seven days. The initial particle size was predictably greater at lower levels of Poloxamer, although the polydispersity indices were all very low. After 7 days the particle size had increased for all three dispersions. As the particle size for the 0.75% and 1.0%, w/w Poloxamer 407 dispersions were similar, a decision was made to select 0.75% Poloxamer 407 for the final formulation, as a compromise between minimizing Poloxamer content while minimizing effects on particle size.

Table 4
Optimization of sorbitol level to achieve iso-osmolality for intravenous formulations containing oleyl glycerate and oleic acid, with the optimal combination of Poloxamer 407 and sorbitol content highlighted in bold

Poloxamer 407 (% w/w)	Sorbitol (% w/w)	Osmolality (mOsm/kg)
1	0	37.5
1	3	226
1	4	281
0.5	4	237
0.75	4	243
0.75	**5**	**302**
1	5	347

The osmolality of the dispersion was also optimized to within the limits of 280–320 mOsm/kg for iso-osmolality with red blood cells to enable the dispersion to be administered intravenously for subsequent preclinical studies. The osmolality of the dispersion containing 1% Poloxamer 407 was measured to be only 35.7 mOsm/kg. Consequently, increasing levels of sorbitol were added post-manufacture to the dispersions with varying Poloxamer levels described above, and the optimal sorbitol content using 0.75% w/w Poloxamer 407 was found be to 5% w/w sorbitol (Table 4).

To ensure that the presence of the stabilizer (Poloxamer 407) and tonicity agent (sorbitol) did not result in a phase change on dispersion of the particles, further flooding experiments under

Fig. 4. Crossed polarised optical microscopy of phytanyl glycerate (Panel A) and oleyl glycerate (Panel B) flooded with water at 37 °C, and oleyl glycerate/oleic acid/irinotecan flooded with 0.75% Poloxamer 407/5% sorbitol solution at 37 °C (Panel C). In each case the excess aqueous solution is at the bottom left corner indicated by the dark region labelled H_2O, whilst the bright fan-like texture appears indicating that reverse hexagonal phase forms in the presence of excess aqueous medium in each case.

Table 5
Formulation of irinotecan in oleyl glycerate/oleic acid Hexosomes for proposed animal pharmacokinetic studies

Component	% in final formulation (w/w)
Oleyl glycerate	9.4
Oleic acid	0.6
Poloxamer 407	0.75
Irinotecan base	0.5
Water for injection containing 5% w/w Sorbitol	88.75

crossed polarised microscopy were performed after preliminary formulation optimization. The presence of H_{II} phase in excess Poloxamer 407/sorbitol solution was confirmed, as illustrated in Fig. 4, right panel.

A further indication that this formulation would form Hexosomes was that the water content (29.3 ± 0.7%, w/w) of the bulk phase at equilibrium with an aqueous solution containing Poloxamer (0.75%, w/w) and sorbitol (5%, w/w) was very close to that of oleyl glycerate alone in excess water (29.9 ± 1.3%, w/w). This suggests that the internal structure of the bulk phase formed with oleyl glycerate when containing oleic acid and irinotecan at equilibrium with excess aqueous solution is likely to be very similar to oleyl glycerate alone, and hence the reverse hexagonal phase would be retained in the particles on dispersion.

As a consequence of the above solubility, phase behaviour, and formulation optimization efforts, the final formulation in Table 5 was adopted for the preparation of Hexosome dispersions.

3.4. Irinotecan Hexosome dispersions—characterization and stability

A number of attributes of the colloidal dispersion prepared according to the formulation in Table 5 were characterized over time. Particle size, polydispersity and zeta potential were measured over time as indicators of colloidal stability when stored at either 25 or 40 °C. The solution pH, % of drug in lactone form and the content of oleyl alcohol were also measured over time as indicators of chemical stability of the dispersions. SAXS was conducted to confirm particle phase structure, and cryo-TEM was used to characterize the physical morphology of the dispersed particles.

The particle size of the dispersion immediately after preparation was 188.6 ± 0.9 nm with a polydispersity index of 0.044 ± 0.011 for three separate samples (mean ± S.D). Over the 53 days storage at 25 and 40 °C, there was no visually apparent degradation of the dispersions at either storage temperature. Fig. 5 illustrates the effect of storage over time on the particle size and polydispersity index. The particle size and polydispersity index were found to increase gradually over time, and to a greater extent for the sample stored at 40 °C. The particle size of the dispersions increased to approximately 250 and 280 nm when stored at 25 and 40 °C, respectively. The stability of the dispersions was considered acceptable given the long storage time, and that the size range was still acceptable for IV

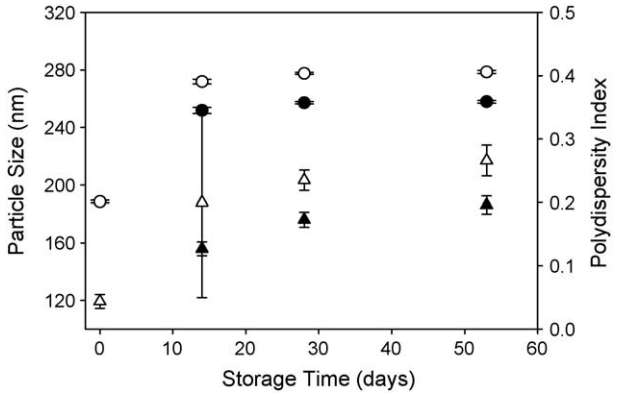

Fig. 5. Particle size (circles) and polydispersity (triangles) of the Hexosome dispersion containing irinotecan over time when stored at 25 °C (filled symbols) and 40 °C (open symbols) (mean ± S.D., $n = 3$).

administration, for preclinical studies. The polydispersity index had also increased to approximately 0.20 and 0.28 at 25 and 40 °C, respectively. For samples stored at 25 °C the measured zeta potential for the particles dropped from approximately −40 to −20 mV, and pH appeared to drop concurrently with zeta potential from 6.6 to 4.4 (Table 6).

The cryo-TEM images in Fig. 6, obtained 7 days after preparation of the irinotecan dispersion revealed the presence of hexagonal shaped particles, however there were also some larger structures without hexagonal faceting, or partly hexagonally faceted 'hybrid' particles. Some of these particles show concentric stripes evident on part or all of the surface of the particles. This is especially apparent in the large worm-like structure in Panel D, where the body has the concentric stripe texture. The worm-like structure in Panel C shows the concentric stripes for the body of the structure, but clearly displays a hexagonally faceted head. It is tempting to suggest that the concentric stripes may be due to the presence of lamellar (bilayer) liquid crystal rather than hexagonal phase, however this is not reflected in the SAXS results. SAXS revealed a scattering pattern consistent with hexagonal phase for this dispersion (data not shown). Importantly, there was an absence of peaks that could be assigned to lamellar phase, suggesting that at least the

Table 6
Influence of storage temperature and time on dispersion pH, zeta potential, % of irinotecan retained in the lactone form (as a total of irinotecan), and the production of oleyl alcohol for a dispersion of irinotecan-loaded Hexosomes prepared from the formulation in Table 5

Storage time (days)	pH	Lactone (%)	Oleyl alcohol (%)	Zeta potential (mV)
25 °C				
0	6.6	94	0.2	38.1 ± 2.0
14	5.7	97	1.2	25.6 ± 1.3
28	5.1	98	1.5	24.5 ± 1.1
53	4.4	99	1.6	21.1 ± 1.0
40 °C				
0	6.6	94	0.2	38.1 ± 2.0
14	4.6	97	1.2	22.0 ± 0.8
28	3.8	99	3	20.1 ± 0.4
53	3.5	100	4	16.5 ± 0.6

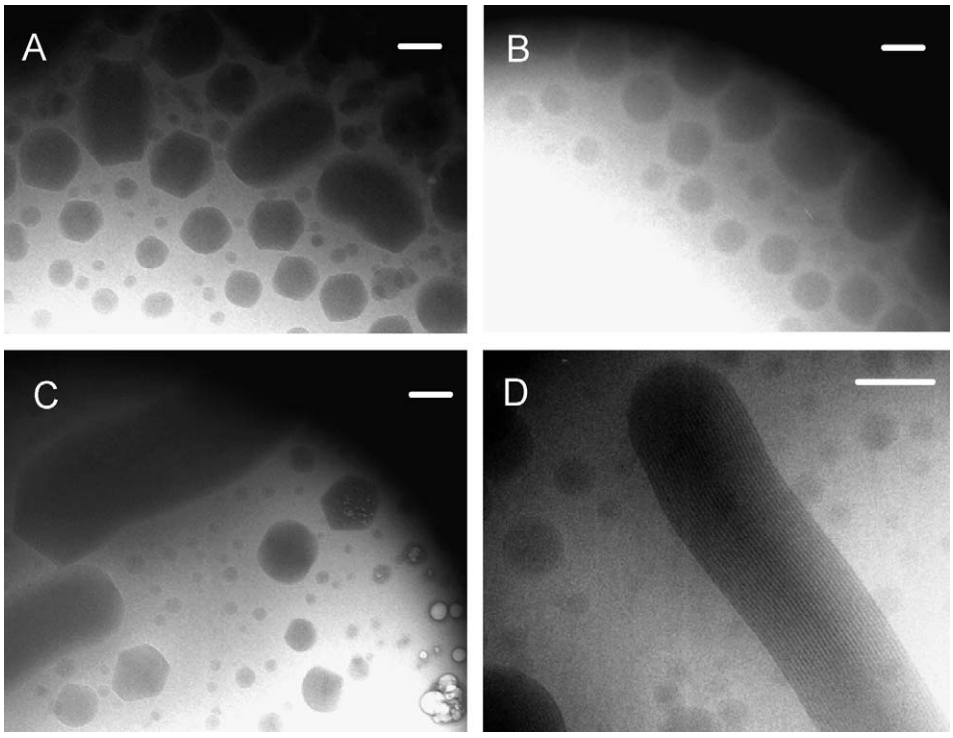

Fig. 6. Cryo-TEM images taken 7 days after preparation of irinotecan Hexosome dispersion. Panels A through D are images of the same sample from different sections of the grid. The white bar represents 100 nm in each case.

majority of the particles comprised a hexagonal phase internal structure.

Immediately after manufacture the ratio of irinotecan in lactone:carboxylate form was 94:6. The work of Rivory et al. (1994) has shown that after 3 h at neutral pH, the lactone form had dropped to only 30% of total irinotecan. For the dispersion prepared in this study, after 3 h at 25 °C the lactone:carboxylate ratio remained high at 89:11. Furthermore, using pressure ultrafiltration it was determined that the ratio of particle bound to free drug was 94:6, thus it is apparent that it is predominantly the free drug component that has converted to the carboxylate form on storage.

Table 6 reveals that when stored at 40 °C the oleyl alcohol content rose by up to 4% of the total lipid in the dispersion. On extended storage, the lactone:carboxylate ratio rose from the initial value of 94:6–99:1 and 100:0 at 25 and 40 °C, respectively. This is consistent with the reduction in pH which favours the equilibrium towards the lactone form of irinotecan.

3.5. Irinotecan release from non-dispersed bulk H_{II} phase versus Hexosomes

Almost identical release profiles of irinotecan were observed from the bulk non-dispersed oleyl glycerate reverse hexagonal phase with and without oleic acid (Fig. 7, Panel A). The data were plotted as % released versus square root of time to emphasise the diffusion-controlled release of irinotecan through the liquid crystalline matrix (Higuchi, 1967). The release curve of irinotecan from oleyl glycerate alone has been published previously by these authors (Boyd et al., 2006). The linear fit of the data, which are an indication of diffusion dependence in these systems, provided correlation coefficients of 0.988 and

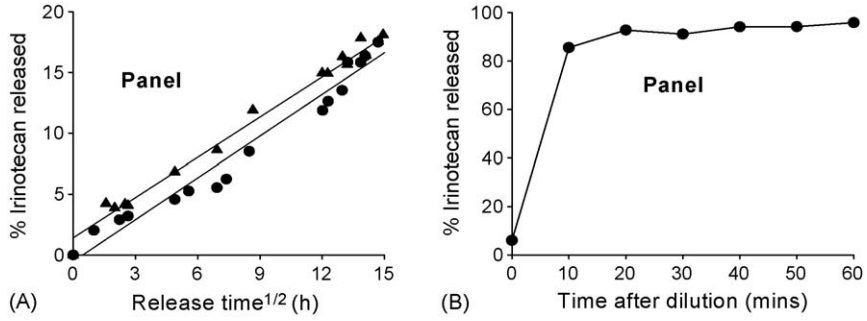

Fig. 7. Panel A: release of irinotecan from bulk reverse hexagonal phase prepared from oleyl glycerate alone (triangles) and oleyl glycerate plus 6% oleic acid (circles) at 37 °C. Lines are the linear regression for each set of data. Panel B: release of irinotecan from OG + OA Hexosomes by pressure ultrafiltration. The line is intended only as a guide to the eye.

0.972 for the oleyl glycerate alone and oleyl glycerate + oleic acid matrices, respectively. This result suggests that oleic acid merely acts to enhance the solubility of irinotecan in oleyl glycerate, but does not significantly affect the release rate or mechanism of release of irinotecan into the aqueous release medium.

Release of irinotecan from Hexosomes was measured using pressure ultrafiltration. Subsequent to extensive dilution of the Hexosome dispersion to create a sink condition, release of irinotecan from the Hexosomes was essentially instantaneous (Fig. 7, Panel B), which agrees well with previous work conducted with Cubosomes loaded with lipophilic drugs (Boyd, 2003). Dispersion of the bulk phase down to sub-micron dimensions increases the diffusion-controlled drug release rate by orders of magnitude, resulting in an apparent burst release of drug on dilution.

4. Discussion

The recently discovered glycerate-based surfactants have been shown to produce Hexosomes when dispersed using high shear methods in the presence of a suitable stabiliser (Poloxamer 407) (Fong et al., 2005) in direct analogy to GMO-based Cubosomes. We thus had a desire to demonstrate the utility of glycerate Hexosomes in the formulation of a problematic drug for which there may be benefits in maintaining chemical stability and possibly providing sustained release.

Irinotecan is an anticancer drug which is highly effective in the treatment of metastatic colorectal cancer. Irinotecan displays a pH dependent equilibrium between its active lactone and inactive carboxylate forms, with rapid conversion to the carboxylate form occurring at neutral pH (Rivory et al., 1994). As a consequence, the currently marketed product Camptosar®, containing irinotecan for infusion, is formulated at a low pH of 3.5 in order to maximize the lactone content at the time of intravenous infusion. However it is anticipated that dilution with infusion liquids and the time required for slow intravenous infusion, often 1.5 h in the clinical setting, will result in conversion of significant proportions of the irinotecan to its inactive form. Minimizing this conversion may be beneficial in facilitating lower effective dose regimes with shorter infusion times.

These studies have shown that the formulation of irinotecan in glycerate-based Hexosomes can improve the retention of irinotecan in the lactone form at near neutral pH. The particle size of the Hexosomes measured using PCS illustrated that the dispersion would be suitable for intravenous administration.

The glycerate surfactants produced in this study are esters of glyceric acid, and are susceptible to hydrolysis, resulting in the formation of the long chain fatty alcohol and glyceric acid. The rate at which this occurs will be dependent on the storage conditions, pH, and temperature. The presence of oleyl alcohol at levels greater than 25% (w/w) in oleyl glycerate is known to induce a phase change from H_{II} to L_2 phase in the presence of excess water (Boyd et al., 2006), however, for the dispersion stored at 40 °C for 53 days, only 4% of the oleyl glycerate had been hydrolysed to oleyl alcohol (Table 6). The longer term hydrolysis of oleyl glycerate observed in these studies by production of oleyl alcohol, and subsequent reduction of solution pH due to glyceric acid, could be alleviated in a commercial product by lyophilization of the Hexosome dispersion. Hydrolytic stability would then only be of concern for the relatively short period of time between reconstitution and administration.

The burst release of drug on dilution of the dispersions is not surprising, based on previous studies of submicron particles using the pressure ultrafiltration method (Magenheim et al., 1993). Whilst it would have provided an additional benefit to the use of these carriers for irinotecan, the ability of the formulation to maintain the drug in its active lactone form at neutral pH is sufficiently compelling for the planning of future intravenous disposition studies in an animal model.

Nanostructured materials such as the liquid crystalline particles described in this publication offer the formulator access to a range of properties not necessarily accessible using conventional materials. In this case the ability to incorporate relatively high levels of lipophilic drug, whilst maintaining the chemical stability of irinotecan in its active form offers some promise for the use of these materials in drug delivery. As the pool of available materials that form these structures grows, the greater the range of properties and potential applications for these delivery systems will become apparent.

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