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Research Paper

Phytantriol and glyceryl monooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems for poorly water soluble drugs I. Phase behaviour in physiologically-relevant media

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Abstract

Objectives The potential utility of liquid crystalline lipid-based formulations in oral drug delivery is expected to depend critically on their structure formation and stability in gastrointestinal fluids. The phase behaviour of lipid-based liquid crystals formed by phytantriol and glyceryl monooleate, known to form a bicontinuous cubic phase in excess water, was therefore assessed in physiologically-relevant simulated gastrointestinal media.

Methods Fixed composition phase studies, crossed polarised light microscopy (CPLM) and small angle X-ray scattering (SAXS) were used to determine the phase structures formed in phosphate-buffered saline, simulated gastric and intestinal fluids in the presence of model poorly water soluble drugs cinnarizine, diazepam and vitamin E acetate.

Key findings The phase behaviour of phytantriol in phosphate-buffered saline was very similar to that in water. Increasing concentrations of bile components (bile salts and phospholipids) caused an increase in the lattice parameter of the cubic phase structure for both lipids. Incorporation of cinnarizine and diazepam did not influence the phase behaviour of the phytantriol- or glyceryl monooleate-based systems at physiological temperatures; however, an inverse hexagonal phase formed on incorporation of vitamin E acetate.

Conclusions Phytantriol and glyceryl monooleate have the potential to form stable cubic phase liquid crystalline delivery systems in the gastrointestinal tract. In-vivo studies to assess their sustained-release behaviour are warranted.

Keywords cubic phase; glyceryl monooleate; liquid crystal; phytantriol; sustained release

Introduction

Co-administration of poorly water soluble drugs with lipids often improves the oral bioavailability of the drug by a number of mechanisms, including maintaining the drug in a persistent solubilised state, delaying gastric emptying, promoting lymphatic transport pathways (thereby bypassing first-pass hepatic metabolism) and attenuating the activity of efflux proteins on the surface of the enterocytes.[1]

The presence of dietary or formulation lipids can stimulate the release of bile salts, phospholipids and digestive enzymes in the gastrointestinal tract. Subsequent emulsification and digestion results in the incorporation of lipid digestion products (primarily monoglycerides and fatty acids) into bile salt and phospholipid mixed micelles.[2–4] The presence of these micelles increases the solubilisation capacity of the gastrointestinal tract for poorly water soluble drugs. Hence, lipid vehicles are an increasingly popular formulation option for drug molecules whose bioavailability is compromised by poor aqueous solubility and/or poor dissolution behaviour.

The formation of liquid crystalline (LC) structures at the surface of oil droplets due to self assembly of the products of triglyceride digestion has been observed during the process of lipid digestion.[5–7] Poorly-soluble amphiphilic lipids often self-assemble to form ordered liquid crystalline structures in the presence of water.[8–10] The type of LC phase formed is influenced not only by global factors such as temperature and water content but

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also by the local effective shape of the molecules themselves. Commonly encountered phases in lipid-based LC systems include the lamellar (L_α), bicontinuous cubic (Q_2) and inverse hexagonal (H_2) phases. These structures are often stable to dilution in excess water, meaning that they can provide a persistent structure and not transform to e.g. micellar structures on dilution.

Several Q_2 and H_2 liquid crystalline structures have been studied as potential sustained-release systems *in vitro*.[8,10] In particular, the amphiphilic dietary lipid glyceryl monooleate, which forms a Q_2 LC (*Pn3m* structure) in the presence of excess water and at physiological temperatures (37°C), has been demonstrated to sustain the release of hydrophilic, amphiphilic and lipophilic drugs *in vitro*.[8,11] However, in-vivo glyceryl monooleate did not provide a sustained-release effect for cinnarizine, a poorly water soluble drug, after oral administration to rats.[12] The digestibility of the glyceryl monooleate molecule in the gastrointestinal tract environment was suggested to be a major factor in the reduced sustained-release effect. In contrast, a poorly digested glyceryl monooleate analogue, oleyl glycerate, which is less readily digested, showed significantly prolonged sustained release.

An alternative polar lipid, phytantriol, has been shown to exhibit similar phase behaviour to glyceryl monooleate despite their differing chemical structures (Figure 1). A bicontinuous cubic phase is also formed by phytantriol in excess water,which has been shown to provide sustained release of incorporated hydrophilic molecules *in vitro*.[13,14] The complete absence of an ester group precludes the potential for lipolysis to influence phase behaviour, pointing to its potential use as a lipid vehicle for oral sustained-release applications.

An understanding of the response of the LC structure formed by phytantriol to the physicochemical environment in the gastrointestinal tract is important for determining the potential of phytantriol as a sustained-release lipid vehicle for oral administration. To date the effect of aqueous solutions representative of physiological media on the phase behaviour of phytantriol has not been reported.

Consequently, in this study, the phase behaviour of phytantriol and glyceryl monooleate in biorelevant fluids has been compared using in-vitro phase identification techniques. The solubility of three candidate model lipophilic drugs, cinnarizine, diazepam and vitamin E acetate, in phytantriol and glyceryl monooleate was determined and the effect of the presence of drug on LC nanostructure assessed. The three selected drugs represented commonly employed lipophilic model compounds and were chosen with a view to their use in subsequent bioavailability studies. Their chemical structures and key chemical properties are in Table 1.[15–19] Phase studies were undertaken using crossed polarised light microscopy (CPLM) and small angle X-ray scattering (SAXS).

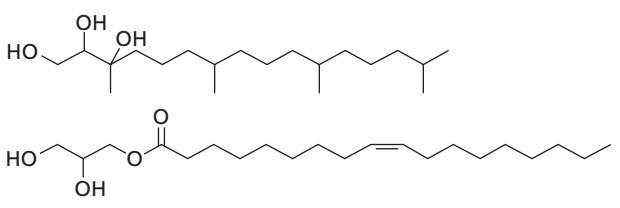


Figure 1 Chemical structure of phytantriol (top structure) and glyceryl monooleate (bottom structure).

Materials and Methods

Materials

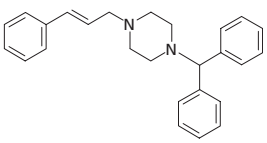
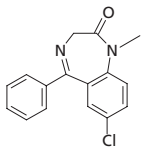
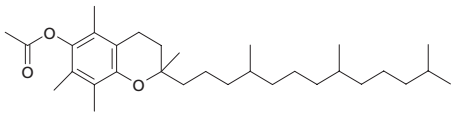
Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was sourced from BASF (Washington, NJ, USA). The initial water content of phytantriol was determined to be less than 0.034% w/w (using Karl Fischer titration). Glyceryl monooleate (in the form of Myverol 18-99) was kindly donated by Kerry Scientific (Norwich, NY, USA), and had a glyceryl monooleate content of 60.9% w/w (monoolein water content of ≤1%).[20] Both lipids were used as obtained without further processing or purification. Model bile salt solutions were prepared using either egg yolk lecithin (consisting of approximately 60% phosphatidyl choline by dry weight) or L-α-lysophosphatidyl choline (LPC) (consisting of approximately 99% LPC by dry weight) (Sigma Co, St Louis, MO, USA). Sodium taurodeoxycholate (NaTDC), cinnarizine, diazepam and α-tocopherol acetate (vitamin E acetate) were obtained from Sigma Co. (St Louis, MO, USA). Sodium hydrogen orthophosphate and potassium dihydrogen orthophosphate were purchased from BDH Chemicals (Kilsyth, Victoria, Australia). Sodium chloride, sodium acid phosphate, 1 M hydrochloric acid and 1 M sodium hydroxide solutions (used in pH adjustments of buffers and solutions) were obtained from Ajax Chemicals (Auburn, New South Wales, Australia). Ammonium dihydrogen orthophosphate was from BDH (Poole, Dorset, UK). All other chemicals were of AR quality. Acetonitrile (Ajax Chemicals, New South Wales, Australia) was of HPLC grade and used as received. Ethanol (99% purity) was obtained from CSR distilleries (Yarraville, Victoria, Australia). Water was obtained from a Millipore Milli-Q filtration/purification system (Billerica, MA, USA).

Solubility studies

The solubilities of cinnarizine, diazepam and vitamin E acetate in phytantriol or glyceryl monooleate were investigated to determine appropriate concentrations for phase behaviour studies, and to assess their suitability as model drug compounds for subsequent oral bioavailability studies. Excess drug was mixed with molten phytantriol or glyceryl monooleate at 37°C, centrifuged at 14 000*g* for 30 min, and supernatant samples (20 mg) were periodically collected (1, 2, 5, 10 and 12 days). Cinnarizine and diazepam content was determined using high performance liquid chromatography (HPLC). Vitamin E acetate was miscible with both lipids at 10% w/w, hence HPLC was not conducted. Equilibrium solubility was deemed to have been achieved when a variation of less than ±5% of the drug concentration was observed between two consecutive time points.

The HPLC method for cinnarizine was adapted from published assays.[21–23] The chromatography was conducted on a C_{18} 5 μm, 150 × 3.9 mm Symmetry RP column (Waters, Milford, MA, USA) and a 7 μm, 15 × 3 mm Brownlee RP guard column (Alltech Associates, Deerfield, IL, USA). The column was coupled to an autosampler (Model 717 plus), a Model 610 fluid unit, Model 600 fluid controller (Waters,

Table 1 Physicochemical properties of the selected model lipophilic drugs cinnarizine, diazepam and vitamin E acetate

	Molecular weight (g/mol)	Aqueous solubility (mg/ml)	Lipid solubility (mg/g)	Partition coefficient (log P)	Chemical structure
Cinnarizine	368.5	$<1.5 \times 10^{-4}$[a]	27.0[a]	5.8[b]	
Diazepam	284.7	0.06[a]	18.3[a]	2.9[c]	
Vitamin E acetate	472.7	<0.1[d]	–	12.2[e]	

[a]Kaukonen *et al*.[15]; aqueous solubility determined in digestion buffer (pH 7.5) and lipid solubility determined in soybean oil. [b]Belsner *et al*.[16]; octanol/water partition coefficient. [c]Taillardat-Bertschinger *et al*.[17]; octanol/water partition coefficient. [d]Sweetman[18]; aqueous solubility in water. [e]Cooper *et al*.[19]; octanol/water partition coefficient of vitamin E.

Milford, MA, USA) and a RF-10A XL fluorescence detector supplied by Shimadzu (Shimadzu Corp., Kyoto, Japan). The detector was set at excitation and emission wavelengths of 249 and 311 nm, respectively. Data was recorded and integrated using Empower 2 personal chromatography data software (Waters, Milford, MA, USA). The mobile phase comprised 50 : 50 v/v ammonium dihydrogen orthophosphate solution (20 mM) : acetonitrile and was eluted at 1 ml/min at room temperature. An injection volume of 50 μl was used with the cinnarizine peak eluting at approximately 5.7 min during the 10-min run. Diazepam was analysed using the same HPLC system as cinnarizine, with the exception that a Waters 486 UV detector (wavelength 230 nm) was used and the mobile phase consisted of 50 : 50 v/v acetonitrile : water. The diazepam peak eluted at 4.7 min within a run time of 10 min. Both assays were validated within acceptable limits of precision and accuracy ($\pm$15% ($\pm$20% at the lower limit of quantitation (LOQ))).[24]

Preparation of simulated gastrointestinal fluids

Simulated gastric fluid (SGF) was 0.1 M hydrochloric acid (pH 2). For simulated intestinal fluids, two model bile solutions were prepared, consisting of NaTDC and either phosphatidyl choline or LPC to simulate 'fasted' intestinal fluids (SIF) containing undigested (phosphatidyl choline) and digested (LPC) phospholipid, respectively. SIF-PC therefore consisted of 1.25 mM phosphatidyl choline and 5 mM NaTDC dissolved in 45 ml phosphate buffer (pH 6.9) containing 101 mM disodium hydrogen orthophosphate, 26.7 mM sodium acid phosphate and 78.7 mM sodium chloride. SIF-LPC was identical to SIF-PC with the exception of the substitution of 1.25 mM phosphatidyl choline for 1.25 mM LPC. Each SIF was prepared by dissolving 1.25 mM either phosphatidyl choline or LPC in 5 ml chloroform in a round-bottom flask. The chloroform was removed by rotary evaporation and a solution of NaTDC and 6 mM sodium azide in phosphate buffer added to the flask. The mixture was equilibrated for at least 12 h under constant stirring in the dark to allow solubilisation of the phosphatidyl choline/LPC film into the bile salts–phosphate buffer solution.

Fixed composition phase studies and crossed polarised light microscopy

Due to the variability in reported phase behaviour for phytantriol LCs in aqueous media, the phytantriol + phosphate-buffered saline (PBS) phase diagram was first established for comparison with previously reported phase diagrams[13,25] and the more complex simulated gastrointestinal fluids. PBS comprised 16.8 mM disodium hydrogen orthophosphate, 1.4 mM potassium dihydrogen orthophosphate and 145.8 mM sodium chloride dissolved in Milli-Q and adjusted to pH 7.40 ($\pm$0.01) with 1 M HCl/NaOH. Eleven 1-g samples consisting of phytantriol and 0, 2, 7, 11, 16, 18, 22, 27, 30, 35 and 40% w/w PBS were prepared in 1-ml glass ampoules. Ampoules were centrifuged at 3500*g* for 15 min at 22°C, and flame sealed under nitrogen gas. Samples were equilibrated at 22°C for four weeks and centrifuged periodically at 3500*g* for 30 min. After four weeks the samples were observed under crossed polarised filters. This process was repeated at 37, 40, 50, 60 and 70°C and observations were continued at each temperature until no further changes in phase behaviour were noted.

The ability of phytantriol and glyceryl monooleate to form LC phases in the presence of drug and simulated gastrointestinal fluids was assessed by CPLM. Samples of phytantriol containing cinnarizine and diazepam at their saturated solubility in the lipid (7 and 24 mg/g, respectively) and vitamin E acetate at subsaturated levels (10% w/w) were

prepared. Although the equilibrium solubilities of cinnarizine and diazepam in glyceryl monooleate were higher than those in phytantriol, lipid solutions of cinnarizine and diazepam in glyceryl monooleate were prepared at an equivalent concentration to those for phytantriol (7 and 24 mg/g, respectively) to permit comparison of the effect of the same concentration of model lipophilic drug on the phase behaviour of glyceryl monooleate as for phytantriol. A small drop of each lipid solution (±drug) was placed between a microscope slide and cover-slip. Simulated gastrointestinal fluid was applied to the edge of the cover slip, which, drawn by a capillary effect, flooded the sample, and penetrated into the lipid droplet, in turn establishing a concentration gradient between the surrounding simulated gastrointestinal fluid and the lipid solution. The sample was observed at 25, 37, 50, 60 and 70°C with the temperature increased at a rate of 10°C/min between each temperature interval and maintained for 3 min before observation. The CPLM equipment comprised an Axiolab E light microscope with crossed polarising filters (Zeiss, Oberkochen, Germany) and an HFS 91 hot stage with a TP 93 temperature programmer (Linkham, Surrey, UK). Observations were made under the microscope at 150× magnification and images were recorded using a Canon Powershot A70 digital camera (Canon, Tokyo, Japan).

Phases were identified by differences in birefringent textures and changes to the rigidity of the phase boundary formed at the interface between the lipid droplet and the surrounding excess fluid (as an indication of viscosity). Of particular interest was the LC phase formed at the boundary between lipid and excess fluid as this was expected to be representative of the phase that would co-exist with excess gastrointestinal fluid *in vivo*.

Small angle X-ray scattering

SAXS was used to confirm CPLM observations of phase identity and to provide a deeper understanding of the effect of increasing concentrations of salt and the simulated gastrointestinal media on the nanostructure of phytantriol and glyceryl monooleate LC phases. To ensure rapid equilibration with the surrounding media, the LC phases were prepared as dispersed emulsion-like systems (the likely state of a lipid formulation under intense shearing in the stomach soon after administration). The phytantriol and glyceryl monooleate dispersions consisted of 10% w/v of lipid ± drug. The lipid ± drug was added to a 1% w/v solution of Pluronic F127 in water. The mixture was ultrasonicated (Misonix XL 2000, Misonix, NY, USA) in a pulsing mode (2-s pulse interrupted by 2-s pauses for 30 min) to assist in the dispersion of the LC. The dispersion appeared as a uniform opaque white mixture with no visible signs of aggregates. The particle size of the dispersed LC systems was measured using a Malvern Instruments Nano-ZS Zetasizer (Malvern, Worcestershire, UK) at 37°C, revealing a particle diameter of 342 ± 18 and 188 ± 9.7 nm for dispersed phytantriol and glyceryl monooleate LCs, respectively, with a polydispersity index of <0.2, indicating that uniform dispersions had been prepared.

SAXS was undertaken on a Bruker Nanostar system with pinhole collimation to ensure precision point geometry. The instrument source featured a copper rotating anode (0.3-mm filament) with cross coupled Göbel mirrors operating at 45 kV/100 mA, providing Cu-Kα radiation with a wavelength of 1.54 Å. The optics and sample chamber were under vacuum to prevent parasitic air scattering. Samples were analysed in 2-mm fine glass capillaries (Charles Supper, MA, USA), sealed airtight with wax and placed in Peltier temperature controlled holders (located in the SAXS instrument) and maintained at 37°C (±0.1°C) to simulate physiological temperatures. SAXS measurements (i.e. scattering patterns) were acquired over 30 min, with LC structures identified by indexing the peaks in the I vs q plot derived from radial integration of the scattering patterns. Specifically these systems form the bicontinuous cubic phases (Q_2) with the *Pn3m* or *Im3m* space groups, with peaks spaced at $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$ and $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{10}$, respectively. Systems that had converted to inverse hexagonal phase (H_2) showed peaks with the spacing 1, $\sqrt{3}$, $\sqrt{4}$. For the cubic phases, the lattice parameter α was derived from the equation:

$$a = d\left(h^2 + k^2 + l^2\right)^{1/2} \quad (1)$$

where the Bragg reflections are annotated using Miller indices hkl and d is the distance between the reflecting planes, defined by Bragg's law $d = 2\pi/q$.

Phytantriol and glyceryl monooleate phase behaviour in the presence of increasing salt concentrations (up to isotonic levels) were determined. The concentration of NaCl present in both saline (154 mM) and PBS (137 mM) used in this study were comparable with levels of Na^+ (142 mM) and Cl^- (126 mM) (the most predominant ions) measured in fasted human jejunal fluid, thus providing a good reflection of the osmolality of fasted gastrointestinal fluids.[26] Hence, to investigate the effect of physiologically relevant concentrations of salts on phytantriol and glyceryl monooleate phases, 10-fold concentrates of PBS and saline (9% w/v sodium chloride) were mixed into blank phytantriol and glyceryl monooleate LC dispersions to provide salt concentrations at 25, 50, 75 and 100% of 154 mM. The added salt solutions did not exceed 10% v/v of the dispersion composition to avoid significant dilution of the lipid components.

Dispersed samples of phytantriol and glyceryl monooleate were prepared as previously described in both fasted (1.25 mM phosphatidyl choline and 5 mM NaTDC) and an intermediate concentration (between fasted and fed conditions) (2.5 mM phosphatidyl choline and 10 mM NaTDC) of SIF. Simulated fed concentrations of SIF (5 mM phosphatidyl choline and 20 mM NaTDC) were not evaluated as the concentrations of bile components were found to fully solubilise the dispersed particles. Control samples without simulated intestinal components were prepared and all dispersions were filled and sealed in 2-mm glass capillaries and equilibrated at room temperature (22°C) for two weeks before SAXS analysis.

Statistical analysis

SPSS for Windows (Version 15.0, SPSS, Chicago, IL, USA) was used to statistically analyse differences in the SAXS data using a one-way analysis of variance with Tukey's multiple comparison, with statistical significance assumed when $P \leq 0.05$.

Results

Solubility of model lipophilic drugs in phytantriol and glyceryl monooleate

The solubilities of cinnarizine in phytantriol and glyceryl monooleate were 7.7 ± 0.1 and 29.9 ± 0.7 mg/g, respectively. Diazepam was significantly more soluble (23.6 ± 0.1 and 47.2 ± 3.4 mg/g in phytantriol and glyceryl monooleate, respectively) and vitamin E acetate solubility exceeded 10% w/w solubility in both phytantriol and glyceryl monooleate. However, CPLM observations (Tables 2 and 3) revealed that phytantriol + 10% vitamin E acetate and glyceryl monooleate + 10% vitamin E acetate formed H_2 and L_2 phases, respectively, rather than cubic phase. Therefore, the effect of addition of vitamin E acetate in greater amounts than 10% w/w was not investigated.

Phytantriol and glyceryl monooleate phase behaviour

Phytantriol + phosphate-buffered saline phase diagram

Observations of samples with fixed composition between crossed polarising filters in a water bath were conducted between 22 and 70°C for preliminary phase determination. At PBS concentrations below 5% w/w and throughout the experimental temperature range, free flowing non-birefringent samples were observed (Figure 2), suggesting an L_2 (inverse micellar) phase was present. At 7% w/w PBS and room temperature (22°C), birefringence and slightly higher viscosity were evident, indicating either the lamellar (L_α) or inverse hexagonal (H_2) phase was present. Above 37°C the sample appearance changed from birefringent to isotropic with similar viscosity to the 5% w/w sample, suggesting reversion to the L_2 phase. Between 20–30% w/w PBS at 22°C an isotropic, highly viscous LC phase was present, indicative of a Q_2 phase. Between 30–60% PBS the highly viscous LC phase co-existed with excess PBS. At temperatures above 60°C viscous isotropic samples became birefringent and appeared less viscous. Subsequent CPLM observations confirmed this to be the inverse hexagonal phase (H_2). At 70°C all samples were isotropic and flowed readily, suggesting conversion to L_2 phase.

Influence of biorelevant media on phytantriol and glyceryl monooleate phase behaviour using crossed polarised light microscopy

CPLM experiments reflect the situation expected to occur as fluid penetrates the phytantriol droplet in the gastrointestinal tract. The phase behaviour of phytantriol + PBS, determined using CPLM (Table 2) was similar to that using fixed composition samples and followed the $Q_2 \rightarrow L_\alpha \rightarrow L_2$ phase progression between excess PBS and lipid with decreasing PBS concentration at 37°C (as demonstrated in the photomicrograph in Figure 3). A Q_2 phase was observed up to 50°C in contact with excess PBS, beyond which $Q_2 \rightarrow H_2$ and $H_2 \rightarrow L_2$ transitions were observed on heating to 70°C (as represented by the broken grey arrow in Figure 2). Based on CPLM results and observations of the phase samples described above, a pseudo-binary partial phase diagram for phytantriol + PBS is presented in Figure 2.

Table 2 Liquid crystalline phases observed adjacent to the excess solution phase boundary using crossed polarised light microscopy when phytantriol or phytantriol plus cinnarizine, diazepam or vitamin E acetate were in direct contact with excess simulated gastrointestinal fluids

System	Temperature (°C)	Liquid crystal at excess simulated gastrointestinal fluid			
		PBS	SGF	SIF-PC	SIF-LPC
Phytantriol	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2	Q_2	Q_2	Q_2
	60	H_2	Q_2/L_2	Q_2/H_2	Q_2
	70	L_2	L_2	L_2	L_2
Phytantriol + cinnarizine	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2/H_2	Q_2	Q_2/H_2	Q_2
	60	H_2/L_2	Q_2/L_2	H_2/L_2	H_2/L_2
	70	L_2	L_2	L_2	L_2
Phytantriol + diazepam	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2	Q_2	Q_2	Q_2
	60	H_2/L_2	L_2	Q_2/L_2	Q_2/L_2
	70	L_2	L_2	L_2	L_2
Phytantriol + vitamin E acetate	25	H_2	H_2	H_2	H_2
	37	**H_2/L_2**	**H_2/L_2**	**H_2/L_2**	**H_2/L_2**
	50	H_2/L_2	H_2/L_2	H_2/L_2	H_2/L_2
	60	L_2	H_2/L_2	L_2	L_2
	70	L_2	L_2	L_2	L_2

The liquid crystal phases observed at the physiologically relevant temperature of 37°C are shown in bold. Simulated gastric fluid (SGF) = 0.1 M HCl; Simulated intestinal fluid-phosphatidyl choline (SIF-PC) contains 1.25 mM phosphatidyl choline and 5 mM sodium taurodeoxycholate (NaTDC); simulated intestinal fluid-L-α-lysophosphatidylcholine (SIF-LPC) contains 1.25 mM LPC and 5 mM NaTDC.

Table 3 Liquid crystalline phases observed adjacent to the excess solution phase boundary using crossed polarised light microscopy when glyceryl monooleate or glyceryl monooleate plus cinnarizine, diazepam or vitamin E acetate were in direct contact with excess simulated gastrointestinal fluids

System	Temperature (°C)	Liquid crystal at excess simulated gastrointestinal fluid			
		PBS	SGF	SIF-PC	SIF-LPC
Glyceryl monooleate	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2	Q_2/H_2	Q_2	Q_2
	60	Q_2/H_2	H_2/L_2	Q_2	Q_2
	70	H_2	L_2	H_2	H_2
Glyceryl monooleate + cinnarizine	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2/H_2	Q_2	Q_2/H_2	Q_2/H_2
	60	H_2	H_2/L_2	H_2	H_2
	70	H_2	H_2/L_2	H_2	H_2
Glyceryl monooleate + diazepam	25	Q_2	Q_2	Q_2	Q_2
	37	**Q_2**	**Q_2**	**Q_2**	**Q_2**
	50	Q_2	Q_2	Q_2	Q_2
	60	H_2	L_2	Q_2	Q_2
	70	H_2	L_2	Q_2	H_2
Glyceryl monooleate + vitamin E acetate	25	H_2	H_2	H_2	H_2
	37	**H_2**	**H_2**	**H_2**	**H_2**
	50	H_2	H_2	H_2	H_2
	60	H_2	L_2	H_2	H_2
	70	H_2	L_2	H_2	H_2

The liquid crystal phases observed at the physiologically relevant temperature of 37°C are shown in bold. Simulated gastric fluid (SGF) = 0.1 M HCl; Simulated intestinal fluid-phosphatidyl choline (SIF-PC) contains 1.25 mM phosphatidyl choline and 5 mM sodium taurodeoxycholate (NaTDC); simulated intestinal fluid-L-α-lysophosphatidylcholine (SIF-LPC) contains 1.25 mM LPC and 5 mM NaTDC.

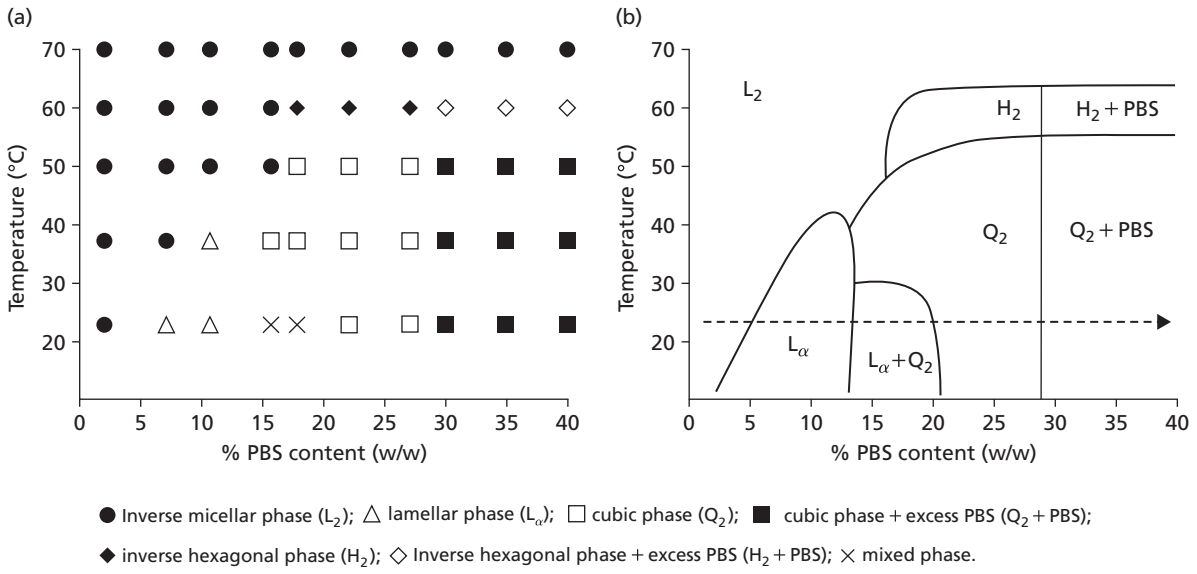


Figure 2 Pseudo binary partial phase diagrams representing the phase behaviour of phytantriol as a function of increasing phosphate-buffered saline content as determined by equilibrium phase samples and crossed polarised light microscopy. Individual data from phase samples were used to construct (a) and the resulting phase diagram is schematically represented in (b). Broken arrow represents the concentration gradient observed in the CPLM studies in Figure 3. PBS, phosphate-buffered saline

The results of the CPLM studies are summarised in Tables 2 and 3. In the case of phytantriol, the presence of SGF and SIF did not induce a phase change away from the Q_2 phase at temperatures below 50°C. However, above 50°C the presence of SGF and SIF (LPC) resulted in a $Q_2 \rightarrow L_2$ transition without the observation of the H_2 phase. All phytantriol systems exhibited a readily flowing contracted phase boundary at 70°C, suggesting the presence of an L_2 phase. Similarly, the phase behaviour of glyceryl monooleate in excess simulated gastrointestinal fluids (Table 3) was qualitatively the same as that of

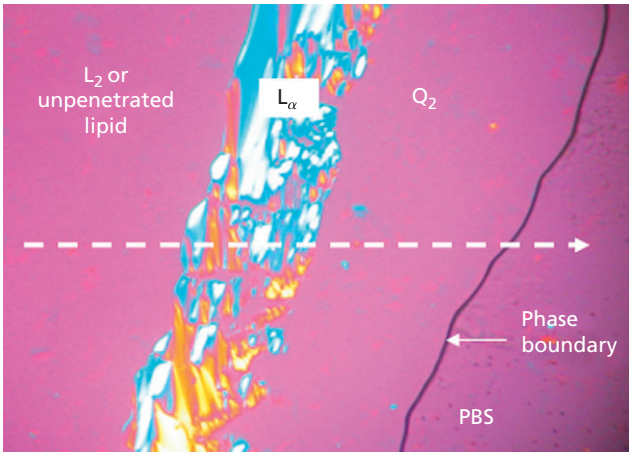


Figure 3 Liquid crystal phases observed using cross polarised light microscopy of a phytantriol droplet in direct contact with excess phosphate-buffered saline. Immediately adjacent to the excess phosphate-buffered saline (PBS) the bicontinuous cubic (Q_2) liquid crystal (LC) is formed as indicated by the deformed phase boundary. Towards the centre of the droplet the PBS concentration decreases and a band of the birefringent lamellar (L_α) phase emerges. The identity of the isotropic phase at low PBS concentration cannot be unequivocally determined, but is most likely to be L_2 (inverse micellar) phase or unpenetrated lipid formulation. Dashed arrow represents compositions with increasing PBS content, analogous to that in Figure 2.

phytantriol, with the Q_2 phase present at temperatures up to 50–60°C in PBS and both SIFs. However, exposure to SGF reduced the $Q_2 \rightarrow H_2$ transition temperature of glyceryl monooleate to 50°C compared with 60°C in PBS. At 70°C, with the exception of glyceryl monooleate in SGF (which was a L_2 phase) all systems exhibited a birefringent H_2 phase.

Influence of drug on phytantriol and glyceryl monooleate phase behaviour using crossed polarised light microscopy

The presence of model lipophilic drugs affected the phase behaviour of phytantriol and glyceryl monooleate to varying degrees when compared with exposure of the lipid alone to excess simulated fluids. A Q_2 LC was present at up to 50°C, when phytantriol + cinnarizine and glyceryl monooleate + cinnarizine were exposed to PBS and the simulated gastrointestinal fluids (Tables 2 and 3). At temperatures above 50°C, cinnarizine had no impact on the phase behaviour of phytantriol + SGF, but did slightly stabilise the glyceryl monooleate + SGF Q_2 phase, raising the $Q_2 \rightarrow H_2$ transition temperature from between 37–50°C to 50–60°C. In contrast, cinnarizine slightly lowered the $Q_2 \rightarrow H_2$ transition temperature of phytantriol in PBS, phytantriol in SIF (phosphatidyl choline and LPC) and glyceryl monooleate in SIF (phosphatidyl choline and LPC) to 50°C compared with 60–70°C in the absence of cinnarizine.

The effect of diazepam was very similar to that of cinnarizine, with a Q_2 phase observed on exposure to simulated gastrointestinal fluids up to 60°C with phytantriol and 50°C with glyceryl monooleate. Interestingly, when phytantriol + diazepam and glyceryl monooleate + cinnarizine were in the presence of SGF, the Q_2 phase converted directly to an L_2 phase between 50–60°C with no evidence of an intermediate H_2 phase, although this comparative study was conducted with limited temperature resolution at high temperature; it is possible that an H_2 phase may have formed at temperatures at which an observation was not made.

Importantly, in the context of providing the necessary phase behaviour information to support subsequent in-vivo studies, the inclusion of diazepam or cinnarizine, in all four aqueous media, did not induce a change away from the Q_2 phase at 37°C. This indicated that all four lipid drug combinations should form the Q_2 phase after oral administration (at least at early times before the effects of digestion and lipid absorption become significant).

In contrast to cinnarizine and diazepam, the presence of 10% w/v of vitamin E acetate had a significant impact on the phase behaviour of phytantriol and glyceryl monooleate. The $Q_2 \rightarrow H_2$ transition temperature was lowered to such an extent that no Q_2 phase was observed at 25°C (Tables 2 and 3). Instead, an H_2 phase was observed in all simulated fluids at 25°C, with a change to $H_2 + L_2$ coexisting phases at 37°C. In the case of the phytantriol systems, at 70°C all systems were isotropic and free flowing suggesting the presence of an L_2 phase.

Phase behaviour of phytantriol and glyceryl monooleate using small angle X-ray scattering

Effect of increasing salt concentration on phase identity and nanostructure

CPLM provides simple information regarding phase identity; however, it does not provide quantitative detail on phase structural dimensions, and in some cases cannot unambiguously confirm the structure present. Hence, SAXS was used to support the CPLM results for phytantriol and glyceryl monooleate in the biorelevant aqueous media, and to investigate structural trends within the phase structures. To enable rapid equilibration of phase structures the lipids were dispersed in Pluronic F127 solution (PF127). Dispersion in PF127 solution has been reported to retain the phase type exhibited by the bulk nondispersed matrix (although changes to the internal space group for glyceryl monooleate have been reported).[27,28]

For phytantriol dispersed in a 1% w/v solution of PF127, X-ray scattering patterns were consistent with a *Pn3m* cubic space group (peak spacing ratios at $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$) (Figure 4a) and gave a lattice parameter of 69.4 ± 0.2 Å (Table 4). The value of the lattice parameter was consistent with those observed previously in bulk (nondispersed) and dispersed systems (66 and 69 Å, respectively).[13,25] Increasing the concentration of saline and PBS did not result in changes to the *Pn3m* structure of dispersed phytantriol at salt concentrations of up to 154 mM; however, significantly smaller lattice parameters were observed ($P < 0.05$). Nevertheless, the reduction in lattice parameters with increasing salt and PBS concentrations did not induce a deviation from the *Pn3m* structure and was consistent with the Q_2 LC observed in the phase samples (Figure 2) and CPLM studies (Table 2).

For the dispersed glyceryl monooleate systems the *Im3m* phase (peak spacing ratios at $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{10}$) was observed both in water and in increasing concentrations of PBS and

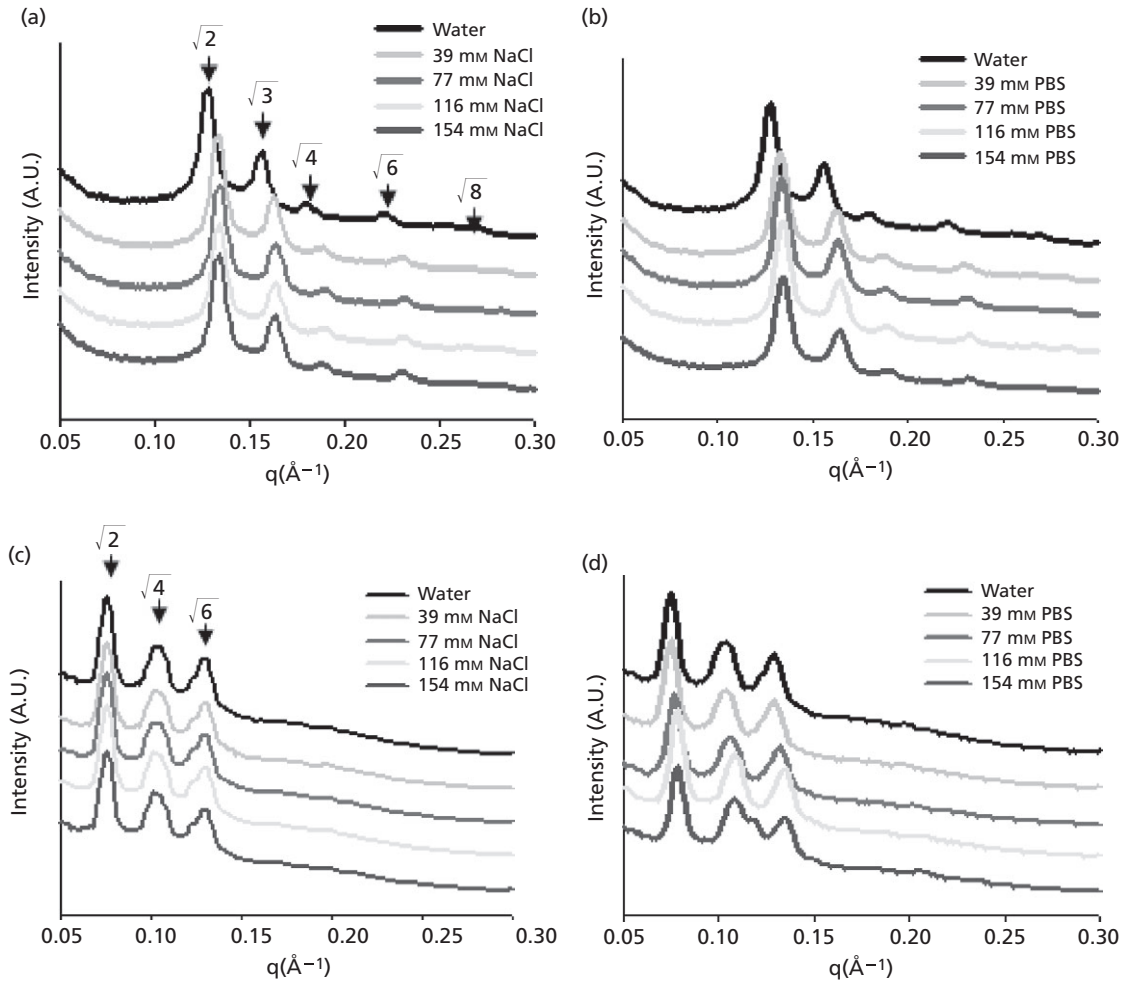


Figure 4 Small angle X-ray scattering profiles for phytantriol and glyceryl monooleate. Dispersions with increasing saline (phytantriol (a) and glyceryl monooleate (c)) or PBS pH 7.4 (phytantriol (b) and glyceryl monooleate (d)) concentrations at 37°C. Dispersions comprised 10% w/v phytantriol or glyceryl monooleate in the presence of 1% w/v Pluronic F127.

saline (Figure 4c, d), with a lattice parameter of 119 Å in water. The space group agreed well with previously reported results, where the *Im3m* space group was observed in dispersed glyceryl monooleate/PF127 systems.[13,27,28] Increasing both salt and PBS concentrations up to 154 mM did not affect the integrity of the *Im3m* Q_2 phase (Figure 4c, d). For glyceryl monooleate at 154 mM NaCl and PBS, the appearance of a shoulder peak between the second ($\sqrt{4}$) and third ($\sqrt{6}$) Bragg peaks suggested the emergence of another phase (most likely the first peak for an H_2 phase). The position of this small peak was at higher q value for NaCl than PBS indicating a slightly smaller lattice. Nevertheless, the SAXS plots indicated that the glyceryl monooleate systems were still predominantly *Im3m* Q_2 phase, and deviated little from that observed with glycerylmonooleate in water.

The effect of simulated intestinal fluid (phosphatidyl choline) concentration on phytantriol and glyceryl monooleate phase behaviour

Bile salt and phospholipid concentrations in the intestine are expected to change depending on the prandial state on and after oral administration. In turn, changes to bile salt and phospholipid concentration might be expected to alter phase structure of self-assembled amphiphiles such as phytantriol and glyceryl monooleate. Hence, the effect of increasing concentration of bile components on phase structure was investigated. The phytantriol (Figure 5a) and glyceryl monooleate (Figure 5b) Q_2 phase structure was partially retained on exposure to SIF containing bile concentrations representative of the fasted state. However, at higher bile concentrations, Bragg peaks of very low intensity were evident suggesting a phase change to a transparent micellar system with near complete dissolution of the cubic phase structure. This was consistent with previous studies on glyceryl monooleate LCs in excess bile salts solutions.[29] The space group of the phytantriol Q_2 phase on exposure to the micellar solutions changed to the *Im3m* space group. Although not clearly observed in the SAXS profiles (Figure 5a, b) sufficient structure was still detected, allowing for lattice parameters to be determined. Significant changes to the lattice parameter were evident for both glyceryl monooleate and phytantriol systems, increasing from 69.4 ± 0.3 and 128 ± 1.2 Å, respectively (Figure 5c) in water to

Table 4 Phytantriol and glyceryl monooleate phase behaviour by small angle X-ray scattering with increasing concentrations of saline or phosphate-buffered saline

Dispersed system	Liquid crystalline phase	Space group	Lattice parameter (Å)	
			Saline	PBS
Phytantriol in water	Q_2	*Pn3m*	69.4 ± 0.2	69.4 ± 0.2
Phytantriol in 39 mM saline/PBS	Q_2	*Pn3m*	66.5 ± 0.2	66.8 ± 0.4
Phytantriol in 77 mM saline/PBS	Q_2	*Pn3m*	66.0 ± 0.2	66.3 ± 0.3
Phytantriol in 116 mM saline/PBS	Q_2	*Pn3m*	66.2 ± 0.4	66.3 ± 0.3
Phytantriol in 154 mM saline/PBS	**Q_2**	***Pn3m***	**66.2 ± 0.2**	**65.7 ± 0.2**
Glyceryl monooleate in water	Q_2	*Im3m*	119.2 ± 0.6	119.2 ± 0.6
Glyceryl monooleate in 39 mM saline/PBS	Q_2	*Im3m*	118.9 ± 1.7	118.5 ± 0.6
Glyceryl monooleate in 77 mM saline/PBS	Q_2	*Im3m*	118.9 ± 1.7	116.2 ± 0.6
Glyceryl monooleate in 116 mM saline/PBS	Q_2	*Im3m*	118.9 ± 1.7	114.6 ± 0.8
Glyceryl monooleate in 154 mM saline/PBS	**Q_2**	***Im3m***	**119.4 ± 1.2**	**114.8 ± 1.4**

Isotonic salt concentrations are highlighted in bold. PBS, phosphate-buffered saline.

105 ± 0.5 ($P < 0.0001$) and 165 ± 2.1 Å ($P < 0.0001$) in fasted SIF and 164 ± 2.1 ($P < 0.0001$) and 182 ± 5.4 Å ($P < 0.0001$) in intermediate SIF, respectively. The increased lattice parameter was likely to be reflecting swelling of the lattice in response to electrostatic repulsion arising from the partition of bile components into the LC structure.

Discussion

The phase behaviours of phytantriol and glyceryl monooleate have been evaluated in the presence and absence of model gastrointestinal fluids and after incorporation of a range of model poorly water soluble drugs. Studies have been performed to establish the potential for phytantriol and glyceryl monooleate to form cubic liquid crystalline matrices *in vivo* and therefore to assess their potential downstream utility as sustained-release oral drug delivery systems. The effect of components likely to be encountered in the gastrointestinal tract (salts, bile salts and phospholipids at physiologically relevant concentrations) on phase behaviour of phytantriol and glyceryl monooleate was therefore evaluated. In general the components present in model gastric and intestinal fluids induced internal changes in LC structure at elevated temperatures, however changes were not observed at physiological temperatures. The incorporation of lipophilic drugs also induced changes in LC phase behaviour; however, the effect varied between the model poorly water soluble drugs examined.

Effect of salt on the phase behaviour of phytantriol and glyceryl monooleate

SAXS analysis of dispersed phytantriol and glyceryl monooleate Q_2 LCs in PBS and saline revealed *Pn3m* and *Im3m* Q_2 lattice structures, respectively (Table 4). In general, the results agreed with previous observations in water, although the presence of salt induced small alterations in phase boundaries and changes in lattice parameters.[13,30] Reduced swelling was noted for phytantriol dispersed in PBS (Table 4), where the lattice parameter (65.7 Å) was smaller compared with that in water (69.4 Å) ($P < 0.05$). Similar changes were observed with dispersed glyceryl monooleate in PBS ($P < 0.05$). The overall reduction in lattice parameter in the presence of the salts likely reflected the reduced water activity in the aqueous channels due to competitive desolvation; however, the biorelevant concentrations of salts used was not sufficient to cause a transition from the cubic LC structure. The reduced water activity reduced the hydration of the headgroup of the amphiphile, resulting in greater curvature towards the aqueous channels. Reduction in lattice parameter in the presence of various salts has been reported for glyceryl monooleate Q_2 systems.[31,32]

The $Q_2 \rightarrow H_2$ transition temperature of phytantriol and glyceryl monooleate in PBS varied from previously reported results in water. In the case of phytantriol, the transition temperature (60°C) was higher than that initially observed with phytantriol in water (42°C).[25] However, the elevated $Q_2 \rightarrow H_2$ transition temperature for phytantriol in PBS (observed in this study) was similar to that reported recently by Dong *et al*.[33] in water, indicating that the presence of salt was likely not responsible for the high transition temperature.

Effect of simulated gastric fluid on the phase behaviour of phytantriol and glyceryl monooleate

CPLM studies with SGF showed a reduction in the temperature of the $Q_2 \rightarrow H_2$ transition of glyceryl monooleate from 60 to 50°C in SGF (Table 3), suggesting the potential for disruption of the LC phase in the presence of SGF. Although the Q_2 structure was observed at physiological temperatures in this study, $Q_2 \rightarrow H_2$ phase transitions in the presence of acid have been previously reported in glyceryl monooleate systems.[34–36] Unionised fatty acids resulting from the acid catalysed hydrolysis of glyceryl monooleate were proposed to occupy the hydrophobic regions of the LC and to promote the formation of inverse phases. However, the residence time in the gastric environment is expected to be of the order of minutes to hours; therefore, initial formation of the cubic phase in SGF, rather than degradation by the acidic environment, is of primary concern for in-vivo application from a phase behaviour perspective.[37]

Unlike glyceryl monooleate, the phase behaviour of phytantriol in SGF was comparable with that observed in PBS (Table 2). This was consistent with the lack of an

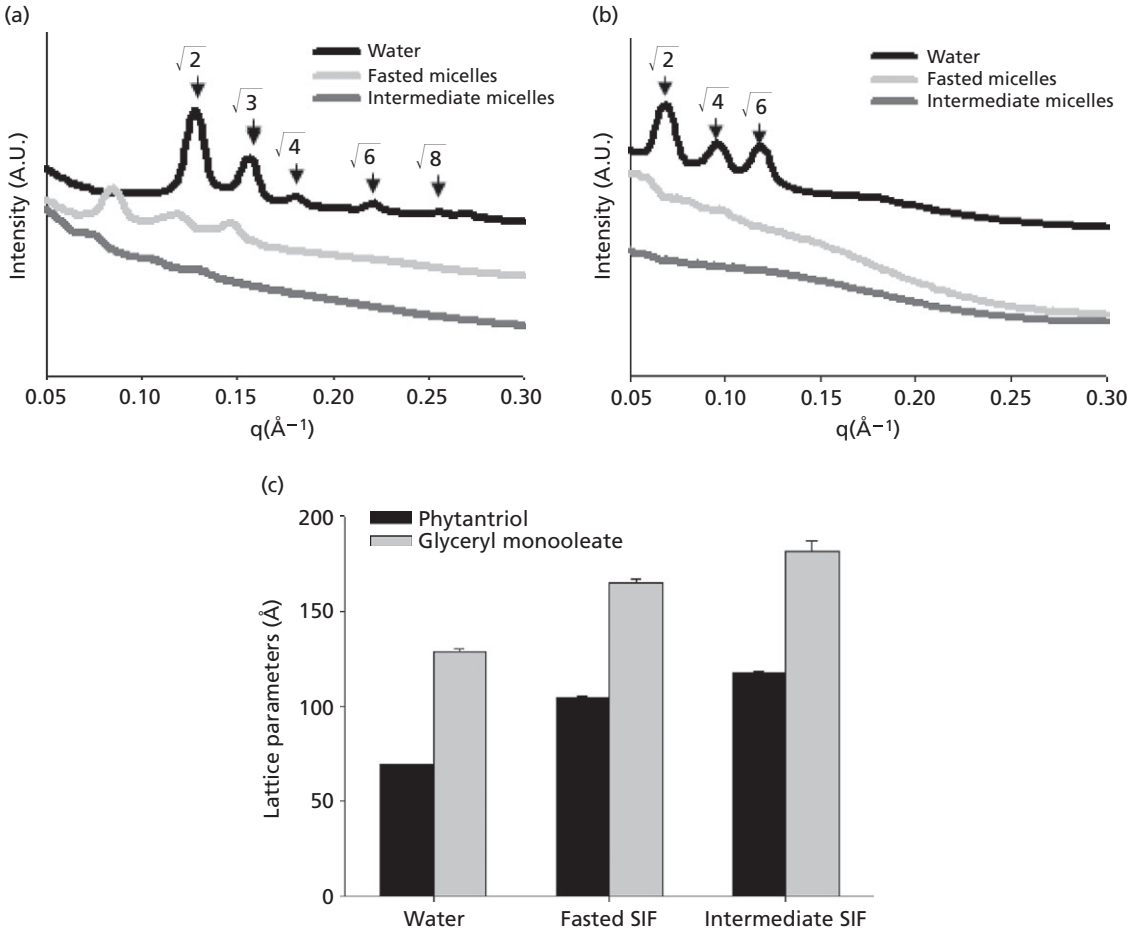


Figure 5 Small angle X-ray scattering profiles for phytantriol and glyceryl monooleate cubosomes and measured lattice parameters. (a) Phytantriol and (b) glyceryl monooleate cubosomes. Measured lattice parameters (c) after prolonged exposure of cubosomes in simulated intestinal fluid (SIF) solutions consisting of (in mM): 1.25 phosphatidyl choline, 5 sodium taurodeoxycholate (fasted) and 2.5 phosphatidyl choline, 10 sodium taurodeoxycholate (intermediate). Samples were equilibrated for two weeks at 25°C before small angle X-ray scattering analysis. Lattice parameter (Å) values are mean ± SD.

acid-labile hydrolysable ester group or other labile group in the phytantriol molecule.

Effect of simulated intestinal fluid on the phase behaviour of phytantriol and glyceryl monooleate

Bile salts and phospholipids, present in the intestinal environment, facilitate the solubilisation of lipid-like molecules during the digestion of fats. Intercalation of endogenous amphiphiles such as bile salt and phospholipid into the phase structure of LC lipid systems might be expected to alter phase behaviour and potentially alter the sustained release effect. Therefore, the interaction of phytantriol and glyceryl monooleate LC systems with bile salts and phospholipids was an important aspect of assessing the likely in-vivo phase behaviour of the systems studied here.

The phase behaviour of phytantriol and glyceryl monooleate at the relatively low concentrations of SIF components used to simulate 'fasted state' conditions (1.25 mM phospholipids, 5 mM bile salts) was comparable with the phase behaviour in PBS at physiological temperatures (Tables 2 and 3). At nonphysiological temperatures the $Q_2 \rightarrow H_2$ transition occurred at higher temperatures for both phytantriol and glyceryl monooleate in SIF when compared with PBS, suggestive of interaction between the intestinal components and the LC nanostructure. Closer investigation using SAXS revealed that increasing bile salts and phosphatidyl choline concentrations resulted in increased lattice parameter dimensions (Figure 5c) and partial solubilisation of the cubic structure (Figure 5a, b). In turn this favoured LC phases with more positive mean curvature (L_α and normal micelles) and was consistent with the increased temperature required to convert the Q_2 phase to the negative curvature H_2 phase. Gustafsson *et al*.[38] noted that the presence of 2–3% w/w of sodium cholate transformed glyceryl monooleate Q_2 phases to swollen L_α and sponge (L_3) phases. Phosphatidyl choline tends to favour the formation of bilayers,which are a common feature in Q_2 and L_α phases.[39,40] Previous studies have shown that when >50% phosphatidyl choline and its analogues were incorporated into a glyceryl monooleate in water system, a $Q_2 \rightarrow L_\alpha$ phase transition occurred.[41,42] LPC forms normal hexagonal and micellar phases in water, suggesting that the incorporation of LPC into the LC systems would favour positive spontaneous

curvature.[39,43] This was consistent with the current CPLM studies, where the temperature required for the transition from Q_2 to more inverse phases (H_2 and L_2) was increased by up to 10°C in phytantriol and glyceryl monooleate in SIF-LPC when compared with in PBS. The increased lattice parameter also suggested insertion of charged bile salts into the bilayer leading to electrostatic repulsion and increased swelling.

Effect of model poorly water soluble drugs on phase behaviour

In addition to the incorporation of intestinal components into the LC structure, the preferential residence of poorly water soluble lipophilic compounds in the hydrophobic region of the LC structure was one factor anticipated to induce changes to phase structures.[44,45] Transition from Q_2 to H_2 phases on addition of drug to the glyceryl monooleate + water system has been reported for acetylsalicylic acid, propranolol, ibuprofen and lidocaine base.[45–47] In this CPLM study, vitamin E acetate induced a Q_2 to H_2 phase conversion at temperatures below 37°C in phytantriol and glyceryl monooleate in PBS. This was significantly lower than was the case when no drug was present (transition temperature of 50–60°C). Dong *et al*.[13] reported that at 37°C phytantriol formed the H_2 phase in the presence of >5% w/w vitamin E acetate and Q_2 phases at 2.5% w/w vitamin E acetate (both in water). It was suggested that the vitamin E acetate molecules filled the voids at the intersection of the hydrophobic tails in the H_2 structure, thereby favouring the formation of the H_2 phase at lower temperatures.[48]

Kumar *et al*.[49] found that the addition of diazepam (27–50 mg/g) into glyceryl monooleate resulted in reduced swelling and drug release from glyceryl monooleate matrices, and suggested that this might be attributed to a change in phase structure, however no supporting phase data was provided. The current CPLM observations of glyceryl monooleate in the presence of 24 mg/g diazepam (Table 3) did not reveal any significant changes in phase structure or transition temperature; however, this does not preclude the possibility that phase changes may be induced at 37°C at higher diazepam concentrations such as those examined by Kumar *et al*.[49] Even when present at saturated concentrations, neither diazepam nor cinnarizine significantly influenced the phase behaviour of phytantriol in the presence or absence of simulated gastrointestinal fluids (Table 2).

Conclusions

This study has shown that the phytantriol and glyceryl monooleate Q_2 LC structure was persistent at physiologically relevant temperatures, in the presence of physiologically relevant salt concentrations and in the presence of SGF and SIF. The model poorly water soluble drugs cinnarizine and diazepam did not have a significant influence on the Q_2 phase structure at the concentrations studied. Hence, it is expected that oral administration of these systems will result in initial formation of the Q_2 phase *in vivo*. The ability of phytantriol and glyceryl monooleate to provide an oral sustained release effect requires in-vivo evaluation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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