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Nanostructure of liquid crystalline matrix determines *in vitro* sustained release and *in vivo* oral absorption kinetics for hydrophilic model drugs

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ABSTRACT

Nanostructured lipid-based liquid crystalline systems have been proposed as sustained oral drug delivery systems, but the interplay between their intrinsic release rates, susceptibility to digestive processes, and the manner in which these effects impact on their application *in vivo*, are not well understood. In this study, two different bicontinuous cubic phases, prepared from glyceryl monooleate and phytantriol, and a reversed hexagonal phase formed by addition of a small amount of vitamin E to phytantriol ($Q_{II\ GMO}$, $Q_{II\ PHYT}$ and $H_{II\ PHYT+VitEA}$, respectively) were prepared. The release kinetics for a number of model hydrophilic drugs with increasing molecular weights (glucose, Allura Red and FITC-dextrans) was determined in *in vitro* release experiments. Diffusion-controlled release was observed in all cases as anticipated from previous studies with liquid crystalline systems, and it was discovered that the release rates of each drug decreased as the matrix was changed from $Q_{II\ GMO}$ to $Q_{II\ PHYT}$ to $H_{II\ PHYT+VitEA}$. Formulations containing ^{14}C-glucose, utilized as a rapidly absorbed marker of drug release, were then orally administered to rats to determine the relative *in vivo* absorption rates from the different formulations. The results showed a trend by which the rate of absorption of ^{14}C-glucose followed that observed in the corresponding *in vitro* release studies, providing the first indication that the nanostructure of these materials may provide the ability to tailor the absorption kinetics of hydrophilic drugs *in vivo*, and hence form the basis of a new drug delivery system.

1. Introduction

Polar amphiphilic lipids that possess a very low aqueous solubility often self-assemble into lyotropic liquid crystalline phases in the presence of excess water (Kaasgaard and Drummond, 2006). Depending upon the nature of the lipid, the presence of additives, and solution conditions the structures formed often include the lamellar (L_α), reversed hexagonal (H_{II}) and reverse bicontinuous cubic phase (Q_{II}). The two or three-dimensional liquid crystalline structure consists of discrete lipidic hydrophobic and aqueous hydrophilic domains and imparts a high viscosity to these materials. Sustained release of amphiphilic, hydrophilic and lipophilic drugs under diffusion control can be achieved from the liquid crystalline matrix (Engström, 1990; Wyatt and Dorshel, 1992; Burrows et al., 1994; Chang and Bodmeier, 1997; Drummond and Fong, 1999; Shah et al., 2001; Kumar et al., 2004; Boyd et al., 2006). Despite the intense interest in these systems, there are still very few studies that demonstrate their usefulness *in vivo*, particularly for the investigation of their application in oral drug delivery.

The most commonly studied material for forming bicontinuous cubic phase in excess water is glyceryl monooleate (GMO, structure in Fig. 1), a common food additive and pharmaceutical excipient (Rowe et al., 2003) that has been shown previously to enhance the bioavailability of co-administered poorly water-soluble drugs (Charman et al., 1993). GMO is also a product of fat digestion, and is itself the subject of lipolysis to produce fatty acids. Digestion of GMO and subsequent breakdown of cubic phase structure, may in part explain the absence of reports of sustained release of drugs using this lipid to form cubic phase upon oral administration. Phytantriol is an excipient frequently used in the cosmetics industry that has also been reported to form bicontinuous cubic phase in excess water at physiological temperature (Barauskas and Landh, 2003). The molecular structure of phytantriol, also illustrated in Fig. 1, does not possess an ester bond, and hence would be likely to retain the cubic phase structure under digestive conditions. The transition to the reverse hexagonal phase occurs at higher

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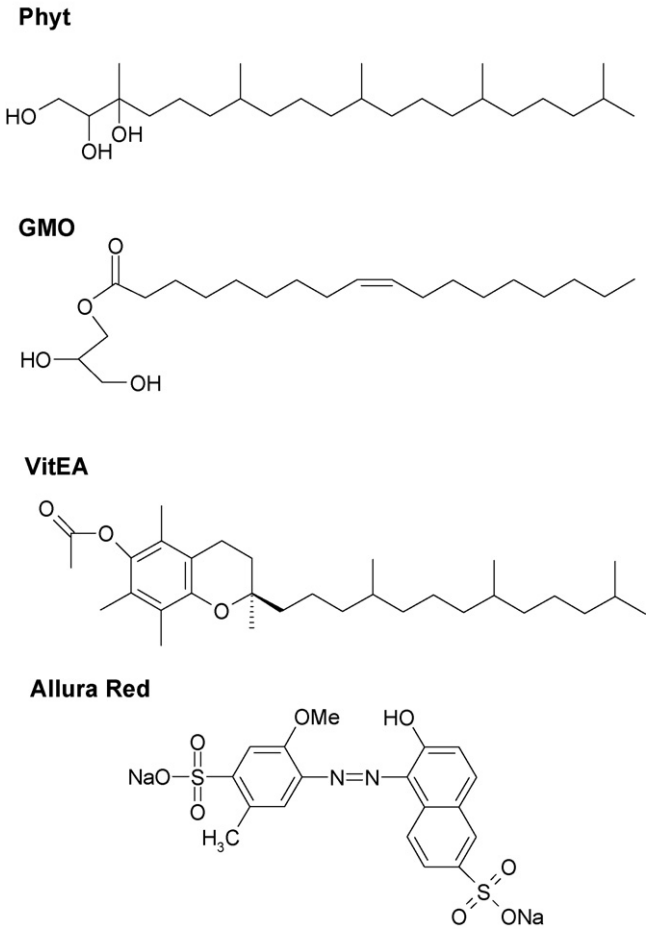

Fig. 1. Chemical structures for phytantriol (PHYT), glyceryl monooleate (GMO), vitamin E acetate (VitEA) and Allura Red.

temperature (Dong et al., 2008), and the transition temperature may be suppressed to 20 °C by addition of 5% (w/w) vitamin E acetate (Dong et al., 2006). Despite the potential benefits that phytantriol-based liquid crystalline matrices may offer as an oral sustained release formulation, there are no reports of the ability of phytantriol liquid crystals to provide sustained release of hydrophilic drugs *in vitro* or *in vivo*.

Consequently, the aims of this study were twofold. Firstly, the ability of phytantriol-based liquid crystalline systems to sustain the release of model hydrophilic drugs has been investigated for comparison with the frequently studied GMO-based cubic phase. Bicontinuous cubic and reverse hexagonal liquid crystalline matrices were prepared using phytantriol, with and without 5% vitamin E acetate respectively, in order to probe the influence of phase structure on drug release. The resulting release data were compared with drug release from the GMO-based bicontinuous cubic phase. Model hydrophilic drugs varying in molecular weight from 180 to 70,000 Da were incorporated into the matrices to probe the impact of drug size on release characteristics from these systems. Second, low viscosity precursors designed to form the viscous liquid crystalline phase *in vivo* were prepared containing ^{14}C-glucose, used as a rapidly absorbed marker of drug release from the formulations. After oral administration of the precursors to rats, the absorption of ^{14}C-glucose was monitored by scintillation counting, to compare the relative absorption kinetics with the *in vitro* release behaviour.

2. Experimental

2.1. Materials

Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was a gift from Roche (Basel, Switzerland). Myverol 18–99 K, was a gift from Kerry Bio-Science (Almere, The Netherlands). Myverol 18–99 K is a commercially available lipid containing a high proportion of GMO and displays phase behaviour very similar to that of pure GMO, and hence is a well-accepted model lipid for the preparation of cubic phase (Clogston et al., 2000).

Allura Red AC (6-hydroxy-5-(2-methoxy-5-methyl-4-sulfophenylazo)-2-naphthalenesulfonic acid disodium salt) is a hydrophilic dye obtained from CHR Hansen Pty. Ltd. (Bayswater, VIC, Australia). Fluorescein isothiocyanate-dextran (FITC-dextran) 4, 20, and 70 were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Radiolabelled ^{14}C-glucose (54.5 mCi/mmol) was from NEN (Boston, MA, USA).

Di-sodium hydrogen orthophosphate, anhydrous was from Univar, APS Ajax Finechem (Auburn, NSW, Australia), and potassium dihydrogen orthophosphate and sodium chloride were from BDH AnalaR, Merck Pty. Ltd. (Kilsyth, VIC, Australia). Sodium azide was from BDH Ltd. (Poole, England). Hydrochloric acid 0.1N and 1N were from AVS Merck Pty. Ltd. (Kilsyth, VIC, Australia) and were used for pH adjustment and to prepare the 0.1 M HCl receptor medium respectively. Water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) purification system (0.05 μS cm^{-1} at 25 °C). Acetonitrile was UV grade from Ajax Finechem (Seven Hills, NSW, Australia). Model bile salt solutions were prepared using either egg yolk lecithin with approximately 60% phosphatidylcholine (PC) by dry weight or L-α-lysophosphatidylcholine (LPC) with approximately 99% L-α-lysophosphatidylcholine by dry weight (Sigma Co., St. Louis, MO). Sodium taurodeoxycholate (NaTDC), was obtained from Sigma Co. (St. Louis, MO). Hydrochloric acid and sodium hydroxide 1 M solutions (used in pH adjustments of buffers and solutions) were obtained from APS Ajax Finechem (Auburn, NSW).

Heparin Injection BP (1000 IU/mL) was purchased from Mayne Pharma Pty. Ltd. (Mulgrave, VIC, Australia). Saline for injection was used from 100 mL polyethylene bags from Baxter Healthcare Pty. Ltd. (Toongabbie, NSW, Australia). Starscint scintillation cocktail was purchased from PerkinElmer (Boston, MA, USA), and 6 mL and 20 mL polypropylene vials for scintillation counting were purchased from Packard Biosciences (Meriden, CT, USA).

2.2. Preparation of liquid crystalline formulations for in vitro drug release studies

Allura Red AC, fluorescein isothiocyanate-dextrans (FITC-dextrans) 4, 20 and 70 and radiolabelled ^{14}C-glucose were chosen as model hydrophilic drugs. Allura Red has a molecular weight of 496 Da and is water soluble with multiple charged groups. It was chosen as a medium molecular weight model drug. FITC-dextrans with average molecular weights of 4000 Da, 20,000 Da, and 70,000 Da were chosen as models for different larger size drugs such as proteins and hormones and also to establish the relationship between drug size and release rates. ^{14}C-glucose has a molecular weight of 180 Da and was chosen as a representative low molecular weight model drug, and in radiolabelled form for analytical purposes in subsequent *in vivo* studies.

Glyceryl monooleate (GMO) and phytantriol (PHYT) were chosen for their ability to form cubic liquid crystalline phase (Q_{II}) in excess water at physiological temperature (Clogston et al., 2000; Barauskas and Landh, 2003). The reversed hexagonal (H_{II}) phase was formed using phytantriol at 37 °C, by addition of 5% (w/w) vitamin E acetate (Dong et al., 2006). This approach provided two

cubic phase matrices using different lipids ($Q_{II\ GMO}$ and $Q_{II\ PHYT}$) and one hexagonal phase matrix ($H_{II\ PHYT+VitEA}$) with which to demonstrate the effect of liquid crystalline phase structure on the release characteristics of hydrophilic model drugs.

The model drugs were dissolved in phosphate-buffered saline (PBS, composition per 1000 mL was 2.38 g Na_2HPO_4, 0.190 g H_2KPO_4, 8.00 g NaCl, 3 mM NaN_3; pH 7.4) at 15.0 mg/mL and the drug solutions were incorporated into the lipids to form the liquid crystalline matrices. From a knowledge of the published phase behaviour for these systems, a composition of 75% lipid and 25% aqueous drug solution was chosen as an appropriate composition close to the excess water boundary to prepare the desired cubic or reversed hexagonal phases, thus minimizing further water uptake on exposure to the receptor medium. Each component was introduced to a small glass vial and the mixtures were heated to approximately 70 °C until flowable, vortexed and centrifuged (5 min, 2800 × *g*) to ensure thorough mixing. The sample was subjected to elevated temperatures for no more than 60 s per heat/mix cycle. This process was repeated three times to obtain homogeneous samples. The mixtures were allowed to equilibrate at 37 °C for a minimum of 48 h.

2.3. Phase behaviour determination

Crossed-polarised light microscopy and visual observation of viscosity were used to confirm that the matrix had adopted the required liquid crystalline phase Q (viscous isotropic and clear) or H_{II} phase (anisotropic, birefringent 'fan-like' texture) (Rosevear, 1954; Rosevear, 1968) both prior to and at the completion of the *in vitro* release studies. Crossed-polarised light microscopy was also used to confirm the identity of phase structures formed on contact with simulated gastric fluid (0.1N HCl in water) (Dressman et al., 1990), simulated fasted intestinal fluid (SIF PC) prior to digestion (5 mM NaTDC/1.25 mM phospholipid mixed micelle solution in PBS) and simulated intestinal fluid (SIF LPC) after digestion of the phospholipid (5 mM NaTDC/1.25 mM lysophospholipid mixed micelles) (Lindahl et al., 1997). In these observations, the H_{II} phase contained 10% vitamin E acetate as it posed a harsher test of the ability of the system to maintain the H_{II} phase in the presence of the aqueous phase components. Each SIF was prepared by dissolving 1.25 mM of either PC or LPC in 5 mL of chloroform in a round bottom flask. The chloroform was removed by rotary evaporation (Rotarvapour RE, Buchi, Switzerland) at room temperature. The model BS (5 mM NaTDC), was subsequently dissolved in PBS and added to the flask. The mixture was equilibrated for at least 12 h under constant magnetic stirring to promote the solubilisation of the PC/LPC film into the BS–phosphate buffer solution. The BS/PC or LPC micellar solutions were used within 5 days of preparation and stored in the dark at 4 °C prior to use. The microscope 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). Temperature was adjusted at 10 °C/min in the heating direction, and held for several minutes before observations of the phase structure formed at the interface with the relevant aqueous 'flooding' solution were made at 25 °C, 37 °C, 50 °C, 60 °C and 70 °C.

Confirmation of liquid crystal phase structure in the presence of glucose as the model hydrophilic drug used in the *in vivo* studies was also undertaken using small angle X-ray scattering (SAXS). SAXS measurements were performed on a Bruker Nanostar SAXS camera, with pinhole collimation for point focus geometry. The instrument source was a copper rotating anode (0.3 mm filament) operating at 45 kV and 110 mA, fitted with cross-coupled Göbel mirrors, resulting in Cu Kα radiation, wavelength 1.54 Å. The SAXS camera was fitted with a Hi-star 2D detector (effective pixel size 100 μm). The optics and sample chamber were under vacuum to minimize air scatter. The sample-to-detector distance was chosen to be 650 mm, which provided a *q*-range of 0.08–0.320 $Å^{-1}$. Samples were loaded into a gel holder sealed with Kapton tape windows, and temperature controlled by use of a Peltier system accurate to ±0.1 °C. Samples were pre-equilibrated at 37 °C for 120 min before making a 30 min exposure. Scattering files were background subtracted and normalised to sample transmission then integrated using Bruker AXS software v4.1.18 to the one-dimensional scattering function $I(q)$, where q is the length of the scattering vector, defined by $q = (4\pi/\lambda)\sin\theta/2$, λ being the wavelength and θ the scattering angle. Peak positions in $I(q)$ versus q plots were indexed to Miller Indices against known space groups. The mean lattice parameter, a, was calculated from the interplanar distance, d, between two reflecting planes, given by $d = 2\pi/q$.

2.4. In vitro release studies

In vitro drug release studies were performed using the USP rotating-basket dissolution apparatus. The receptor medium consisted of 300 mL of PBS at pH 7.4 for studies modelling physiological pH. The release studies for glucose containing formulations were also performed using 0.1 M hydrochloric acid (pH 2) as a representative media for modelling gastric conditions to establish whether low pH in the gastrointestinal environment influences drug release rates in preparation for the *in vivo* study. Samples of the liquid crystalline matrices were constrained in a pre-weighed glass micro-beaker with an approximate volume of 400 μL, and a single open face circular face with fixed surface area of 50.2 mm^2. The micro-beaker was placed in the basket and lowered into the receptor medium, with the basket rotating at 100 rpm to facilitate rapid removal of released drug from the matrix/receptor medium interface. All release studies were performed at 37 °C to model physiological temperature. Due to the potential light sensitivity of the fluorescein-labelled dextrans and Allura Red, the entire apparatus was protected from light for the duration of the studies. At set time points indicated in Fig. 2, samples were removed and stored at −20 °C until analysis. An equivalent volume of receptor medium was replaced to maintain constant volume.

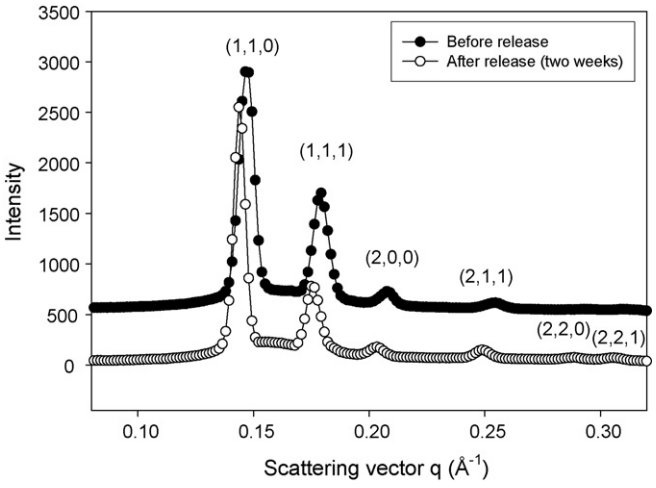

Fig. 2. Small angle X-ray scattering profiles for matrices in excess water at commencement of release and after release for the phytantriol + glucose cubic phase system. Numbers in parentheses indicate the Miller indices (*h k l*) for the reflecting planes in the sample giving rise to the peak in intensity vs. scattering vector. The indices indicated correspond to the cubic phase with *Pn*3*m* spacegroup.

2.4.1. Analytical methods

2.4.1.1. Allura Red. Samples containing Allura Red were analysed using a validated HPLC assay, on an Ultrasphere C_8 5 μm, 4.6 mm × 25 cm column (Beckman, USA). A Waters 717 Plus autosampler coupled to a System Gold, 126 Solvent Module, 168 UV detector system (Beckman, USA) was used with detection wavelength was 500 nm. An injection volume of 100 μL was used and the column was eluted with a mobile phase of 20% acetonitrile, 80% PBS (pH 7.4) at a rate of 1.0 mL/min at room temperature giving a retention time for Allura Red of 3.8 min. Samples were compared to standards of known concentrations (1000 ng/mL, 500 ng/mL, 250 ng/mL, 100 ng/mL, 75 ng/mL, and 50 ng/mL) run before and after the study samples. Intraday and interday assay validation was performed, providing accuracy of 104%, 105%, and 100% and precision of 5.8%, 2.9%, and 2.5% at 50 ng/mL, 500 ng/mL and 1000 ng/mL, respectively.

2.4.1.2. FITC-dextrans. FITC-dextrans (FD-4, FD-20, and FD-70) were analysed using black 96-well assay plates (BMG Polystyrene, Germany) on a FLUOstar OPTIMA platereader (BMG Labtechnologies, Germany). 150 μL of sample was placed into each well and samples were measured in duplicate against standards of known concentrations (50 ng/mL, 75 ng/mL, 100 ng/mL, 250 ng/mL, 500 ng/mL, 1000 ng/mL) at room temperature. The fluorescence method was validated as described for Allura Red, providing accuracy of 94.4%, 101%, and 99.5% and precision of 6.4%, 3.4%, and 2.8% of 50 ng/mL, 500 ng/mL and 1000 ng/mL, respectively.

2.4.1.3. ^{14}C-glucose. ^{14}C-glucose was analysed by scintillation counting. 1 mL of receptor medium was sampled from the dissolution vessel and mixed with 16 mL of scintillation cocktail (Starscint) in a 20-mL scintillation vial for scintillation counting on a Tri-Carb 2800TR, PerkinElmer Liquid Scintillation Analyser. To determine the limit of quantitation of ^{14}C-glucose by scintillation counting, several concentrations of ^{14}C-glucose in PBS were prepared in triplicate from the stock solution (equivalent to 19.5, 39.0, 58.5, 78.0 disintegrations per minute), 100 μL of each concentration was added to 1 mL of scintillation liquid in 6 mL scintillation vials, and the samples vortex mixed and counted. The lowest concentration with precision within 10% and accuracy within 15% was set as the limit of quantitation (LOQ) for all ^{14}C-glucose *in vitro* release samples.

2.4.2. Data analysis for release studies

Previous studies using the geometry in this study (single sided slab of matrix containing dissolved drug) (Clogston and Caffrey, 2005) have shown that liquid crystalline systems display Fickian diffusion-controlled release according to Eq. (1); consequently, the data were also plotted as drug released versus square root of time ($t^{1/2}$).

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad (1)$$

where Q is the mass released per unit area, and C_0 the initial concentration of drug in the matrix, D is the diffusion coefficient for drug in the matrix and t is time of release (Higuchi, 1967).

Interrogation of these plots allows confirmation of release kinetics as first order diffusion-controlled release, evident from a linear relationship between mass released and $t^{1/2}$. The slopes from the individual plots were combined to yield the release rate constant and error, which could subsequently be used to determine the significance of difference between the drug/matrix combinations using *t*-test and one-way analysis of variance (ANOVA). All *in vitro* data are reported as mean ± standard deviation of at least three replicates as indicated in tables and in figures.

2.5. In vivo oral absorption studies

2.5.1. Formulation preparation for in vivo studies

Due to the inherent high viscosity of liquid crystalline phases, a low viscosity liquid crystal precursor system, known to rapidly form Q_{II} or H_{II} phase on exposure to gastric fluids was prepared to enable dosing via oral gavage (Boyd et al., 2006).

The precursor systems were prepared in the same manner as described above for the *in vitro* release samples, except that a lesser amount of aqueous drug solution (10%, w/w) is included, such that the precursor is in the low viscosity L_2 phase region of the lipid/aqueous solution phase diagram. Precursor systems were prepared and equilibrated by mixing on a tube roller at 37 °C for 48 h.

The three liquid crystalline precursors were (i) a cubic phase precursor comprising phytantriol (90%, w/w), and an aqueous solution of glucose (10%, w/w), (ii) a cubic phase precursor prepared from Myverol 18–99 K (90%, w/w) and an aqueous solution of glucose (10%, w/w), and (iii) a reverse hexagonal phase precursor prepared from phytantriol (85.5%, w/w), vitamin E (4.5%, w/w) and an aqueous solution of glucose (10%, w/w). The aqueous solutions used to prepare the precursors contained glucose at 167 mg/mL. A fourth formulation comprising an aqueous solution of glucose in PBS at the same dose of glucose as the precursors was used as a negative control. A fifth formulation was an IV solution of glucose in saline to enable absolute bioavailability calculations. Radiolabelled glucose (1 μCi) was mixed with unlabeled glucose to ensure the same nominal mass of glucose was dosed in each case.

2.5.2. Pharmacokinetic studies

All experimental procedures involving animals were approved by the Victorian College of Pharmacy Animal Ethics Committee, Monash University, Parkville, VIC, Australia. *In vivo* absorption studies were conducted using rats (male, Sprague Dawley, 250–300 g) which were cannulated via the carotid artery using polyethylene tubing 0.96 mm × 0.58 mm (Paton Scientific, Victor Harbour, Australia) under isoflurane anaesthesia as described previously (Lyons et al., 2000). In the intravenous dosing studies, the jugular vein was also cannulated to facilitate dosing of ^{14}C-glucose solution. The cannulas were flushed with heparinised saline (20 IU per/mL) and flame sealed before insertion into a subcutaneous pocket at the back of the neck. The rats were housed in separate cages with water available *ad libitum*. After an overnight recovery period, including a 12 h pre-dose fasting period, the rats were administered the dosing formulation (containing a nominal dose of 20 mg/kg of ^{14}C-glucose, corresponding to an approximate mass dose of 300 mg of liquid crystal precursor formulation) by oral gavage whilst under anaesthesia. For IV studies the rats were administered the dosing solution (containing a nominal dose of 20 mg/kg of glucose in 200 μL saline) by infusion over 2 min into the jugular vein via the in-dwelling cannula, followed by 0.25 mL of heparinised saline to ensure complete infusion of the contents of the cannula.

Blood samples (0.2 mL), obtained from rats dosed with lipid formulations were collected from the carotid artery pre-dose and at 10 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 360 min and 480 min, 24 h, 30 h, 48 h and 54 h post-dosing. For rats dosed with the lipid-free solution formulation, blood samples were also collected at 20 min and 45 min. For intravenous administration, blood samples were collected pre-dose, at the instant of conclusion of the infusion ($t = 0$), at 5 min, 10 min, 20 min, 40 min, 60 min, 90 min, 120 min, 180 min, 240 min, 360 min and 480 min and at 24 h. Blood samples were placed immediately into a tube containing 10 IU of heparin and were centrifuged for 5 min at 10,000 rpm. Plasma (100 μL) was added to 1 mL of Starscint scintillation cocktail and vortexed before analysis by liquid scintillation counting.

The plasma assay was validated for precision (<10%) and accuracy (<20%) to establish the LOQ.

2.5.3. Pharmacokinetic analysis

Data were converted to plasma concentration using the known activity of the glucose used in this study, accounting for dilution and assuming that the radiolabel was still associated with intact glucose (which may or may not be the case, but as glucose was used merely as a rapidly absorbed marker compound, this was sufficient to determine the relative release rates of glucose from the formulation prior to absorption). Plasma concentrations (μg/mL) were normalised to a dose of 20 mg/kg. Pharmacokinetic parameters, including total area under the plasma concentration–time curve (AUC), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}), after administration of all oral formulations were calculated directly from the plasma ^{14}C-glucose concentrations (Shargel and Yu, 1993). To allow a more thorough interrogation of data obtained after oral dosing studies, intravenous dosing studies were also conducted to allow determination of the absolute bioavailability of the model drug (although it should be kept in mind that glucose pharmacokinetics and bioavailability assessment were not a primary aim of this study). Bioavailability (F%) of glucose from the various formulations was calculated using the relation:

$$F\% = \frac{AUC_{oral} \times dose_{IV}}{AUC_{IV} \times dose_{oral}} \times 100$$

Data for each formulation was then presented on a log-linear scale against time to permit visual comparison of terminal elimination behaviour between the various formulations.

Further interrogation of the data to compare rank order of absorption of glucose from the different formulations, as an indicator of release, was by comparison of statistical significance of differences in t_{max} using one-way analysis of variance (ANOVA).

3. Results

3.1. Formulation phase behaviour

The presence of model drug in the liquid crystalline preparations did not affect the phase behaviour in any of the model drug/liquid crystal combinations studied and the phase structure before and after release was preserved. There was no birefringent texture evident for the cubic phase samples prepared with phytantriol or GMO, and the addition of model drugs changed neither the birefringence nor the viscosity of the samples. Representative SAXS scattering profiles are illustrated in Fig. 2, for the phytantriol matrix loaded with glucose before and after the release experiment. The SAXS data for the cubic phase samples for phytantriol and GMO in excess aqueous solution both showed the *Pn*3*m* cubic phase structure was present, consistent with previous studies on phytantriol (Barauskas and Landh, 2003) and GMO alone (Briggs et al., 1996). For the pre-release phytantriol + glucose cubic phase, the lattice parameter was 60.6 ± 0.1 Å, which was slightly lower than the 61.9 ± 0.2 Å for the post-release matrix. Birefringent texture consistent with H_{II} phase was evident in the phytantriol + vitamin E acetate system as expected. The presence of glucose in the aqueous domains of the H_{II} phase gave a lattice parameter of 47.9 Å, consistent with phytantriol + vitamin E acetate in excess water (Dong et al., 2006).

Crossed-polarised light microscopy, summarised in Table 1, confirmed that neither physiologically relevant levels of bile salts and phospholipids/lysophospholipids representative of the environment in the small intestine, nor pH 2 solution representative of the low pH environment of the stomach, induced changes from the phase structure observed for the formulations in PBS at 37 °C, although some differences did become apparent at higher temperatures.

Table 1
Phase structure present on exposure of PHYT (phytantriol alone), GMO or PHYT + VitEA (phytantriol + vitamin E acetate), to various simulated physiological media determined by crossed-polarised light microscopy

Lipid	Temperature (°C)	PBS pH 7.4	SGF (0.1N HCl)	SIF (PC)	SIF (LPC)
PHYT	25	Q_{II}	Q_{II}	Q_{II}	Q_{II}
	37	Q_{II}	Q_{II}	Q_{II}	Q_{II}
	50	Q_{II}	Q_{II}	Q_{II}	Q_{II}
	60	H_{II}	Q_{II}/L_2	Q_{II}/H_{II}	Q_{II}
	70	L_2	L_2	L_2	L_2
GMO	25	Q_{II}	Q_{II}	Q_{II}	Q_{II}
	37	Q_{II}	Q_{II}	Q_{II}	Q_{II}
	50	Q_{II}	Q_{II}/H_{II}	Q_{II}	Q_{II}
	60	Q_{II}/H_{II}	H_{II}/L_2	Q_{II}	Q_{II}
	70	H_{II}	L_2	H_{II}	H_{II}
PHYT + VitEA	25	H_{II}	H_{II}	H_{II}	H_{II}
	37	H_{II}	H_{II}	H_{II}	H_{II}
	50	H_{II}/L_2	H_{II}/L_2	H_{II}/L_2	H_{II}/L_2
	60	L_2	H_{II}/L_2	L_2	L_2
	70	L_2	L_2	L_2	L_2

PBS is phosphate-buffered saline at pH 7.4; SGF represents gastric fluid (0.1N HCl in water); SIF (PC) represents simulated intestinal fluid prior to digestion (5 mM NaTDC/1.25 mM phospholipid mixed micelle solution in PBS); SIF (LPC) represents simulated intestinal fluid after digestion of the phospholipid (5 mM NaTDC/1.25 mM lysophospholipid mixed micelles). Q_{II}, H_{II} and L_2 are viscous cubic phase, reverse hexagonal phase and low viscosity reverse micellar phase, respectively.

3.2. In vitro drug release

In vitro release studies were conducted to determine the influence of the nature of the liquid crystalline matrix on the release behaviour for a series of model hydrophilic drugs. The drug release profiles for all five model drugs (^{14}C-glucose, Allura Red, FD-4, FD-20 and FD-70) from the liquid crystalline matrices are illustrated in Fig. 3, Panels A to E, respectively. Data from the release studies were plotted as percentage released against square root of time to interrogate the release mechanism, to provide an indication of relative release rates and for calculation of diffusion coefficients.

3.2.1. Release into PBS at pH 7.4

In almost all cases, the profiles were essentially linear, indicating that diffusion is the predominant mechanism of drug release, even in the case of release from the $H_{II\ PHYT+VitEA}$ matrix. Importantly, in all cases there was a general trend in decreasing rate of release from the different matrices for each drug, in that release was the most rapid from $Q_{II\ GMO}$, then from $Q_{II\ PHYT}$ and was the slowest from the $H_{II\ PHYT+VitEA}$ matrix.

In the case of glucose (Panel A), >80% of the drug was released from the $Q_{II\ GMO}$ matrix in 2 days, whilst <10% was released from the $H_{II\ PHYT+VitEA}$ matrix over the same time period. Perhaps even more striking was the difference in the rate of release of Allura Red from the various matrices (Panel B); almost 60% of the Allura Red was released in 1 week from the GMO-based system but <2% was released from $H_{II\ PHYT+VitEA}$ even after 2 weeks. Drug release from all three matrices was able to be determined for glucose and Allura Red in Panels A and B. The slower release of FD-4 resulted in approximately half the amount of model drug released compared to Allura Red over the same time frame for the different cubic phase matrices (Panel C), and the release of FD-4 from the H_{II} matrix was too slow to enable the release to be quantified for all three separate samples. Release of FD-20 (Panel D) from the $H_{II\ PHYT+VitEA}$ matrix was too slow to yield any data above the LOQ within the practical experimental period of twelve days, hence only data for release

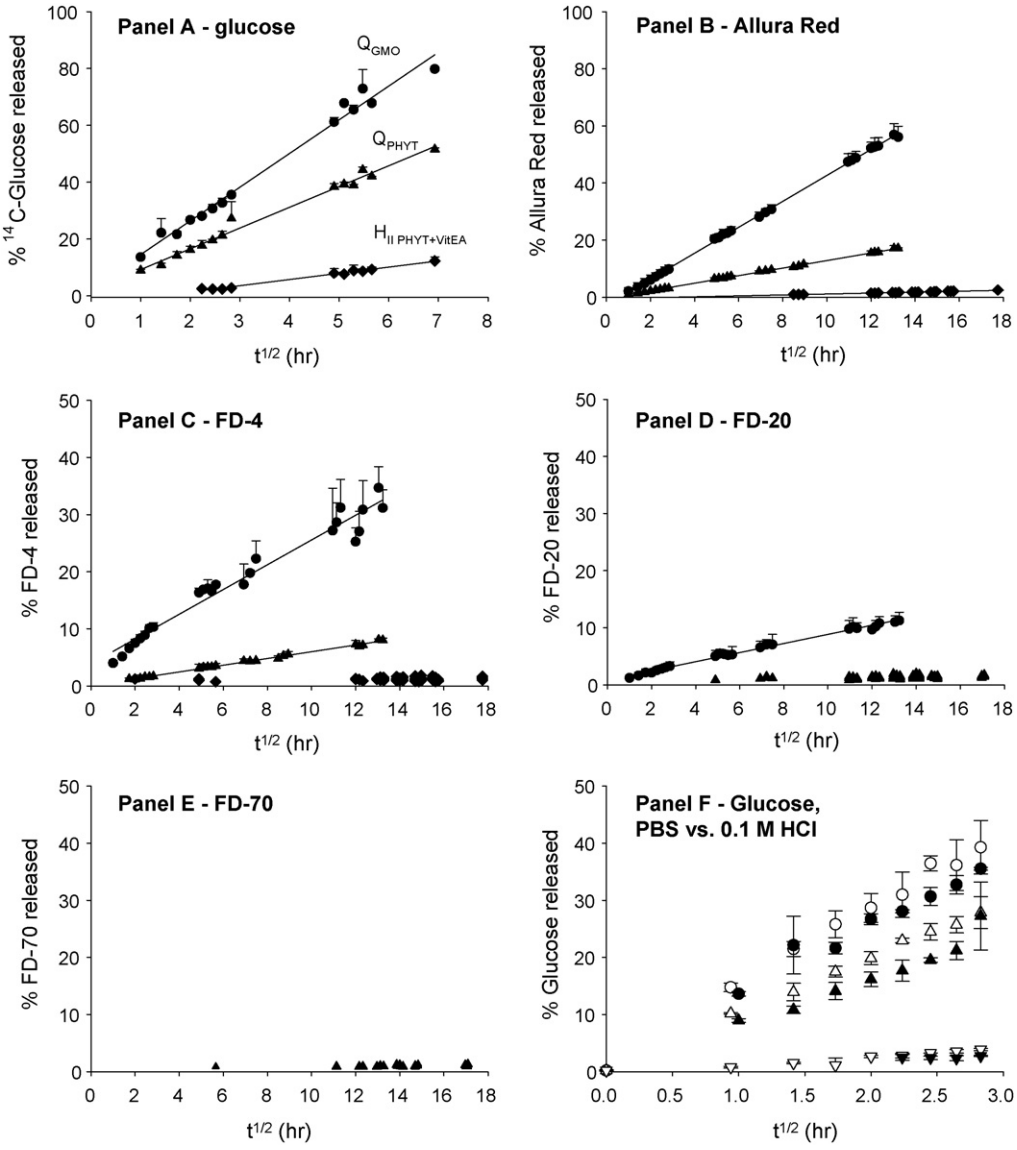

Fig. 3. Release profiles for hydrophilic model drugs from the cubic ($Q_{II\ GMO}$ (●) and $Q_{II\ PHYT}$ (▲)), and reversed hexagonal ($H_{II\ PHYT+VitEA}$ (♦)) liquid crystalline matrices into phosphate-buffered saline, plotted against square root of time. Solid lines indicate linear fits to release profiles. Absence of fit indicates $n < 3$ profiles contain data above the LOQ, otherwise data are mean ± S.D., $n = 3$. Panel A illustrates release data for ^{14}C-glucose; Panel B: Allura Red; Panel C: FD-4; Panel D: FD-20; Panel E: FD-70; Panel F illustrates release of glucose from the matrices (symbols as above) into PBS, pH 7.4 (closed symbols reproduced from Panel A), and 0.1 M HCl, pH 2 (open symbols).

from the two cubic phase samples is presented, and only release from the GMO cubic phase was sufficient to allow quantitation of all three separate release samples. Likewise, the release of FD-70 was too slow from both $Q_{II\ PHYT}$ and the $H_{II\ PHYT+VitEA}$ matrix, hence only quantifiable individual data from $Q_{II\ GMO}$ are presented in Panel E to demonstrate the extreme reduction in release rate for the large model drug.

The FITC-dextran release curves from $Q_{II\ GMO}$ in Panels C and D appear to show two 'phases' of release, with an initial more rapid release phase over the first 10 h, followed by a slightly slower release phase over the following ten days. The reason for this is not clear at this time–temperature control in the apparatus was 37 ± 1 °C, so it appears likely that the dextran may have had a subtle influence on the structure of the liquid crystalline phase which affected the release rate, but not the phase identity. Alternatively, the matrix may not have been fully swollen considering that 25% aqueous phase was used to form the cubic phase matrix, but this did not appear to impact on the Allura Red release profile from $Q_{II\ GMO}$ in Panel B, and was not evident in any of the release profiles from the phytantriol-based systems, further indicating that it was this particular drug type-matrix combination that produced the effect.

3.2.2. Release into 0.1 M HCl at pH 2

The release experiments for glucose from all three matrices were repeated using 0.1 M HCl as the receptor medium, to ensure that the release behaviour would be maintained in a representative gastric environment in preparation for *in vivo* studies. The release data using 0.1 M HCl as the receptor medium are illustrated in Fig. 3 Panel F in open symbols, with the release data using PBS as receptor medium reproduced for direct comparison over the same time frame. The glucose release profiles into 0.1 M HCl closely follow those in which PBS was used as the receptor medium (no significant difference between release profiles for different media; $p > 0.08$ for comparison of slope of release versus square root time profiles between media), indicating that the acidic conditions did

Table 2
Diffusion coefficient ($\times 10^8$ cm^2 s^{-1}) for model hydrophilic drugs in liquid crystalline matrices calculated from release data (mean ± S.D., $n = 3$) at 37 °C

Model drug	MW (Da)	$H_{II\ PHYT+VitEA}$	$Q_{II\ PHYT}$	$Q_{II\ GMO}$
Glucose	180.2	6.6 ± 1.2	73.7 ± 2.8	194.8 ± 0.2
Allura Red	496.4	0.0361 ± 0.0034	2.41 ± 0.07	28.1 ± 1.4
FD-4	4,300	–[a]	0.474 ± 0.033	6.83 ± 0.96
FD-20	21,200	–[a]	–[a]	0.906 ± 0.080

Abbreviations: $Q_{II\ PHYT}$ = phytantriol cubic phase; $Q_{II\ GMO}$ = GMO cubic phase; $H_{II\ PHYT+VitEA}$ = phytantriol + 5% vitamin E acetate reversed hexagonal phase.

[a] Release too slow to enable measurement of quantifiable amount of material for all three runs in 14 days.

not influence the release rate or the release mechanism for the glucose from the liquid crystalline matrices.

3.2.3. Calculated diffusion coefficients

Using Eq. (1) and the fit data for the individual replicate Q versus $t^{1/2}$ release profiles, a diffusion coefficient for drug in each drug-matrix combination was determined, and are presented in Table 2. Comparison of the magnitude of the diffusion coefficient shows that (i) the impact of matrix on diffusion coefficient for individual model drugs follows the general order $Q_{II\ GMO} > Q_{II\ PHYT} > H_{II\ PHYT+VitEA}$, and (ii) the diffusion coefficient of the model drugs was reduced as the molecular weight was increased in all cases. Using one-way ANOVA analysis for each model drug, statistically significant difference was shown between the diffusion coefficients in each matrix in all cases ($p < 0.001$). Similarly, for each individual liquid crystalline matrix, the diffusion coefficient for each of the model drugs within that matrix was also significantly different ($p < 0.05$).

3.3. In vivo absorption studies

The hypothesis governing these studies was that the relative rates of *in vitro* drug release from the different matrices would translate into differences in oral absorption rates *in vivo*. To test this hypothesis ^{14}C-glucose was chosen as the model compound as it is rapidly absorbed and therefore the kinetics of its appearance in plasma would likely be governed by its rate of release from the matrix. In addition to the low viscosity precursors for the $Q_{II\ PHYT}$, $Q_{II\ GMO}$, and $H_{II\ PHYT+VitEA}$ matrices, ^{14}C-glucose was also administered intragastrically in the form of an aqueous solution as a control formulation (positive in terms of drug availability, negative in terms of sustained release).

The mean plasma concentration versus time profiles for ^{14}C-glucose following administration of all five formulations are illustrated as semi-log plots in Fig. 4 Panel A, over the entire sampling duration of 54 h. The terminal elimination kinetics were very similar; it should be noted that glucose is likely to be rapidly distributed and metabolised *in vivo* subsequent to entering plasma, meaning that elimination is not entirely due to excretion of glucose. It should also be noted that metabolism of ^{14}C-glucose will alter the species with which the radiolabel is associated, and hence although the profiles have been plotted against dose normalised ^{14}C-glucose concentrations, the radiolabel may not be strictly in

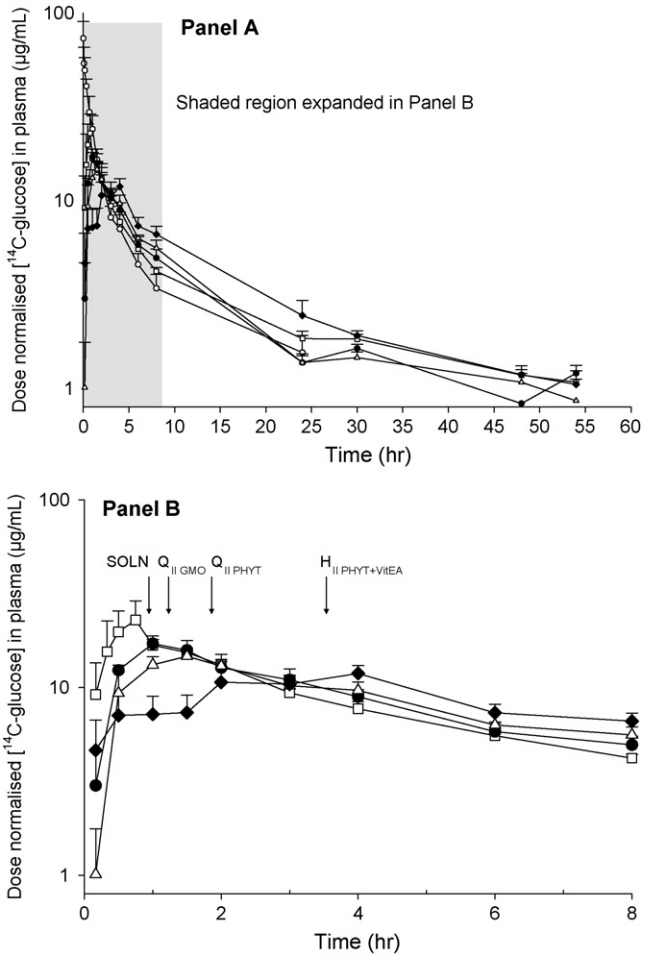

Fig. 4. Normalised plasma concentration profiles for ^{14}C-glucose after administration to rats. Panel A: glucose in plasma after intravenous administration of aqueous solution formulation is denoted by open circles (○). Glucose in plasma after administration of the remaining formulations administered by oral gavage, denoted by aqueous solution (□), $Q_{II\ GMO}$ (●), $Q_{II\ PHYT}$ (Δ), and $H_{II\ PHYT+VitEA}$ (♦). Panel B: plasma concentration profiles truncated to 8 h after oral administration for comparison between the three lipid-based formulations and the oral aqueous solution. Annotations indicate the mean t_{max} value obtained for that formulation (from Table 3). Data are mean ± S.E.M., $n = 4$. Lines in both panels are intended as a guide to the eye only.

Table 3
Pharmacokinetic parameters for ^{14}C-glucose after oral and intravenous administration in rats (dose normalised to 20 mg/kg)

	$H_{II\ PHYT+VitEA}$	$Q_{II\ PHYT}$	$Q_{II\ GMO}$	Solution	IV
AUC (µg/mL h)[a]	226 ± 7.1	244 ± 20	259 ± 30	224 ± 14	185 ± 13
C_{max} (µg/mL)	13.4 ± 1.4	15.2 ± 1.1	17.8 ± 1.2	25.1 ± 5.3	–
t_{max} (h)	3.25 ± 0.48[b,c]	1.88 ± 0.38	1.13 ± 0.13	1.00 ± 0.34	–
F%	90.5 ± 4.5	91.8 ± 7.4	108 ± 22	82.0 ± 7.2	–

Values are given as mean ± S.E.M. ($n = 4$ for oral formulations, $n = 3$ for IV). Abbreviations: $Q_{II\ PHYT}$ = phytantriol cubic phase; $Q_{II\ GMO}$ = GMO cubic phase; $H_{II\ PHYT+VitEA}$ = phytantriol + 5% vitamin E acetate reversed hexagonal phase; solution = ^{14}C-glucose solution in phosphate-buffered saline; IV = intravenous infusion of ^{14}C-glucose in phosphate-buffered saline. AUC = area under plasma concentration vs. time curve; C_{max} = peak plasma concentration; t_{max} = time to reach peak plasma concentration; *F*% = absolute oral bioavailability.

[a] AUC truncated to 54 h. (extrapolation to $t_{inf} < 0.1\%$ of total).

[b] $p < 0.01$ vs. solution.

[c] $p < 0.01$ vs. $Q_{II\ GMO}$.

glucose form, and at long times this is certainly not anticipated to be the case. However, the similarity in the profiles certainly indicates that at long times (>8 h) the formulation type did not appear to influence the subsequent *in vivo* processing of the radiolabel.

The pharmacokinetic parameters derived from each of the profiles (AUC, t_{max}, C_{max} and *F*% (absolute oral bioavailability)) are presented in Table 3. The first point to note is that all of the lipid precursor formulations provided absolute oral bioavailability (*F*%) close to 100%. The Solution formulation had a slightly reduced oral bioavailability, but was not statistically different to that of the other formulations. The values for C_{max} were greatest for the solution formulation, as would be anticipated in light of expected rapid absorption from this dose form, and decreased sequentially in the order solution > $Q_{II\ GMO}$ > $Q_{II\ PHYT}$ > $H_{II\ PHYT+VitEA}$.

In the context of this study it is primarily the t_{max} values in which we are most interested to assess whether the *in vitro* release data was reflected in the temporal absorption behaviour. The time to maximum plasma concentration was only 1 h for the Solution formulation, which was expected to be the most rapidly absorbed formulation as there was no sustained release matrix therefore no mechanism for sustained release. The mean t_{max} for glucose absorption after administration of the various formulations was earliest for the solution formulation (1.00 h), followed by $Q_{II\ GMO}$ (1.13 h) and $Q_{II\ PHYT}$ (1.88 h) and $H_{II\ PHYT+VitEA}$ (3.25 h). For clarity, the profiles for appearance of ^{14}C-glucose from the intragastrically administered formulations have been truncated to 8 h in Fig. 4 Panel B to permit visual comparison of the t_{max} values. There was a significant difference between the t_{max} of the solution and $Q_{II\ GMO}$ dosing formulation when compared to the $H_{II\ PHYT+VitEA}$ formulation using the one-way analysis of variance (ANOVA) at $p < 0.01$. Although the difference in t_{max} for glucose after administration in $Q_{II\ PHYT}$ could not be distinguished statistically from that of $H_{II\ PHYT+VitEA}$ or $Q_{II\ GMO}$, as the annotations in Fig. 4 Panel B indicate, there was a trend towards delayed t_{max} in line with the release rate constant determined for each matrix in the *in vitro* release experiment.

4. Discussion

There were two principal aims for this work, firstly to investigate the controlled release characteristics of phytantriol based liquid crystalline matrices as an alternative to the more extensively investigated GMO system, and second to investigate how the nanostructure of the liquid crystalline materials can be manipulated to alter drug absorption rates after oral administration. Changes in absorption kinetics, after oral administration of glucose in the different liquid crystalline matrices used in this study, generally reflected the differences in *in vitro* release kinetics. Statistically it was not possible to distinguish between $Q_{II\ GMO}$ and $Q_{II\ PHYT}$ *in vivo* with the sampling schedule employed in this study, however future applied studies using actual drug molecules would employ more frequent sampling around the C_{max}, which may permit this distinction to be made without increasing the number of subjects. Nevertheless the primary purpose of the study as a proof of concept to demonstrate control over release using changes in nanostructure *in vitro* and *in vivo* using a model hydrophilic compound has been achieved, and future studies using therapeutically relevant compounds is warranted.

The differences between measured diffusion coefficients for drugs within the matrices, and hence release rates, agreed with the concept that lattice parameter (greatest for GMO-based cubic phase (approx. 100 nm), then phytantriol-based cubic phase (70 nm) and phytantriol + vitamin E acetate hexagonal phase (approximately 40 nm) (Dong et al., 2006)), and hence internal water channel diameter determines the drug diffusion rate for hydrophilic compounds (Clogston and Caffrey, 2005). The magnitude of the diffusion coefficients in GMO were in agreement with previous studies investigating the diffusion of a range of hydrophilic drugs in the GMO cubic phase (Clogston and Caffrey, 2005); for example myoglobin was previously determined to have a diffusion coefficient of 0.40×10^8 cm^2 s^{-1} at 20 °C, compared 0.91×10^8 cm^2 s^{-1} at 37 °C for the similar sized FD-20 in this study (Table 2).

4.1. Utility of nanostructured liquid crystalline matrices in oral delivery

Liquid crystalline matrices such as reversed hexagonal and bicontinuous cubic phase are gaining increasing attention as potential drug delivery systems (Drummond and Fong, 1999) due in part to their potential for sustained and controlled release, however very few reports of *in vivo* assessment of liquid crystalline systems for oral delivery in the literature.

The oral administration of cinnarizine (a poorly water-soluble antihistamine) in oleyl glycerate (OG, a poorly digested H_{II} forming lipid) and GMO illustrated the potential impact of digestibility on these systems (Boyd et al., 2007); however it was shown that the OG matrix provided extended absorption of drug for several days, indicating long residence of the formulation in the GI tract and a poor sink condition *in vivo* inhibiting drug release. Such a dramatic duration of drug absorption was not observed in this study for the phytantriol + vitamin E acetate H_{II} formulation, most likely due to the sink condition in the GIT for glucose. Again using cinnarizine as the model drug, Kossena et al. (2004) investigated the intraduodenal administration of cinnarizine loaded into preformed cubic phase comprising a monolaurin/lauric acid mixture, resulted in a slow increase in the concentration of cinnarizine in plasma compared to a suspension formulation. However the study did not compare absorption rates from different nanostructures, nor was the formulation subjected to the gastric environment making it difficult to compare with the results obtained in the OG study (Boyd et al., 2007) or to those of the current study.

The oral delivery of sodium pamidronate using the H_{II} matrix formed by oleyl glycerate has been disclosed in the patent literature (Boyd et al., 2005). Sodium ^{14}C-pamidronate was administered in a low viscosity precursor formulation, similar to the L_2 phase H_{II} precursor described in this study, to rats by oral gavage and appearance of pamidronate indicated by radioactivity in plasma. The result was a 11-fold increase in AUC for pamidronate compared to an aqueous solution control formulation, thereby demonstrating the potential that these novel formulations may deliver in a clinical setting. Absorption was delayed until 5 h after administration of the liquid crystal precursor and one of two rats showed elevated radioactivity in plasma at 24 h. The effect of liquid crystal nanostructure was not investigated in that study, although it would certainly be of interest to further investigate the impact of nanostructure on pamidronate absorption kinetics with a more extensive protocol.

The oral administration of drugs incorporated into dispersed liquid crystalline particles has also been reported. Cyclosporin, a poorly water-soluble cyclic peptide, has been administered orally in dispersed particles of cubic phase yielding improved bioavailability but not impacting significantly on time to reach peak concentration in plasma (Bojrup et al., 1996). A lack of sustained release in this case may have been attributed to the short diffusional distance required for drug release (Boyd, 2003), and perhaps shorter residence time of dispersed particles in the GIT compared to the bulk lipid formulations used in the present study. The antihypertensive agent omaprilat has been administered orally to rats, and provided a rapid and more pronounced antihypertensive effect compared to drug in suspension, but the pharmacokinetics were not reported, making it difficult to determine whether a sustained release behaviour was evident (Tamayo-Esquivel et al., 2006). The oral administration of

insulin loaded into GMO cubic phase particles provided a hypoglycaemic effect comparable to intravenous administration of insulin over a 6 h period after oral administration (Chung et al., 2002), although the exact mechanism providing the hypoglycaemic effect nor insulin pharmacokinetics were reported and there have been no further reports on this approach in the subsequent 6 years.

Hence there appear to have been no reports in the academic literature demonstrating control over absorption of hydrophilic drugs in lipid-based systems through manipulation of matrix nanostructure.

The rates of appearance of drug in plasma in this study were significantly delayed for the lipid formulations; the usual transit time for orally administered drugs through the gastrointestinal tract is of the order of several hours, and glucose was absorbed for approximately three and a half hours in the case of the H_{II} phase formulation in this study. One alternative explanation for the delay in t_{max} for the lipid formulations is that the presence of the lipid merely slows the gastric emptying rate (Hunt and Knox, 1968), however this hypothesis would not account for the statistical difference between the t_{max} after administration of the $H_{II\ PHYT+VitEA}$ formulation and the $Q_{II\ GMO}$ formulation.

The quantitative absorption from the lipid formulations occurred over a period of only several hours, whilst release of glucose in the *in vitro* testing occurred over days. The emulsifying ability of the stomach is well known, and it is likely that the formulation is at least in part further reduced to emulsion like particles (in the case of these lipids cubosomes and hexosomes (Larsson, 1999)) with overall higher surface area from which drug can diffuse and subsequently be absorbed. However, the release of drugs from very small particles under diffusion control is extremely rapid (Boyd, 2003; Rosenblatt et al., 2007) due to very high surface area, leading to the conclusion that the lipid administered by gavage retains a relatively low surface area for some time after administration to enable release to occur on a relatively slow time scale with discrimination between the structure of the matrices able to be determined. Analogous studies using GMO- and oleyl glycerate-based low viscosity precursors has demonstrated that diffusion-controlled release can be obtained in a similarly designed *in vitro* release test for hydrophilic drugs (Boyd et al., 2006). We plan to progress these studies to a canine model to enable the administration of the dose form in capsules (as would be done in humans), which should clarify any uncertainty surrounding the actual geometry of the administered dose form through the gavage that was necessary for the current studies in rats.

4.2. Phytantriol as a new oral excipient for sustained release

We believe that these studies are the first to utilize phytantriol for oral drug delivery. There has been one report comparing the utility of $Q_{II\ PHYT}$ and $Q_{II\ GMO}$ for improved transdermal absorption of δ-aminolevulinic acid (Bender et al., 2005). Phytantriol may offer a benefit over GMO as a drug delivery excipient due to its non-digestibility, meaning that, unlike GMO (Boyd et al., 2007), the liquid crystalline structure can be retained in the presence of digestive enzymes. The potential of the poorly digested OG to resist the effect of enzymatic degradation and operate as a sustained release excipient *in vivo* has already been shown (Boyd et al., 2007), and further studies along similar lines for phytantriol using poorly water-soluble compounds will be reported in future. The ability to readily manipulate the phase behaviour of phytantriol to modify release kinetics *in vivo* using additives such as vitamin E acetate further make phytantriol an exciting potential new excipient.

In conclusion, the *in vitro* release characteristics of hydrophilic drugs from phytantriol based liquid crystalline structures was established. The results demonstrated that both the liquid crystalline nanostructure and the nature of the lipid itself can be utilized to control the rate of release of hydrophilic drugs of varying molecular weight. The rank order of release from the matrices was reflected in the kinetics of drug absorption after oral administration *in vivo*, indicating the potential for these systems to provide control over hydrophilic drug release after oral administration.

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