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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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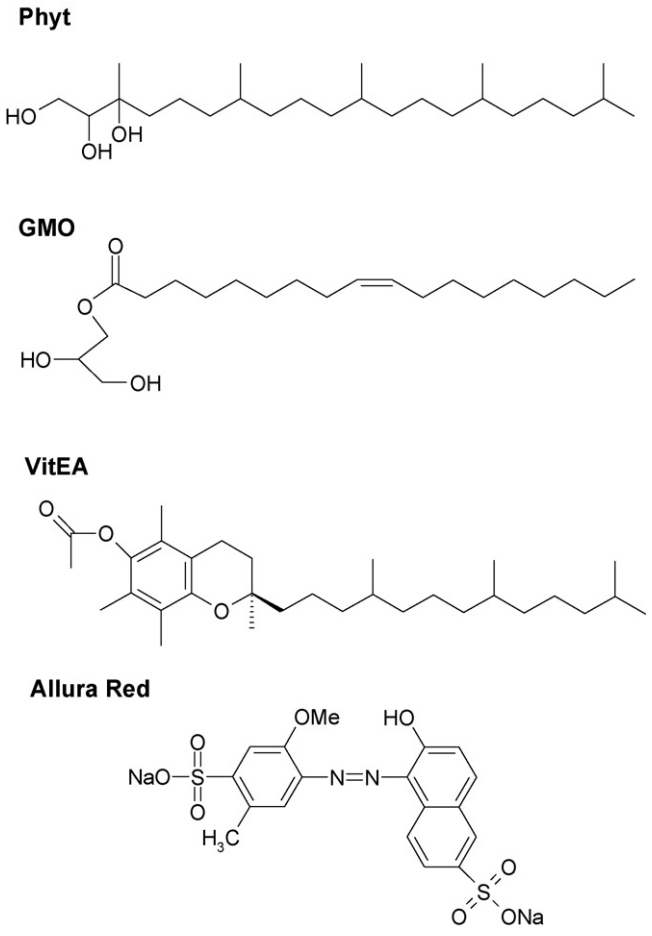

Fig. 1. Chemical structures for phytantriol (PHYT), glyceryl monooleate (GMO), vitamin E acetate (VitEA) and Allura Red.

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