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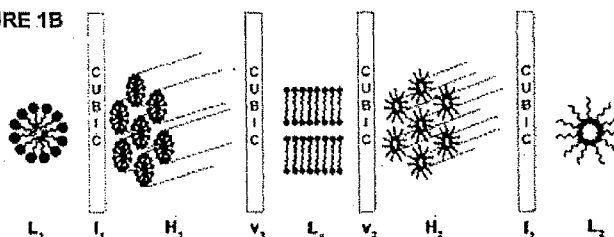
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(54) Title: LIQUID CRYSTAL COMPOSITION AND METHOD OF USE

FIGURE 1B



(57) Abstract: The present invention provides a method, system and composition for controlled compound delivery, the composition comprising a liquid crystal carrier and a compound to be delivered. An external stimulus can be applied to control transition of the liquid crystal from a first phase having a first capacity for retention of the compound to a second phase having a second capacity for retention of the compound. The 'capacity for retention' is the ability of the liquid crystalline matrix to reduce the apparent diffusion rate of the compound within the matrix to less than that in bulk solvent coexisting with the carrier, such as excess water, bodily fluid or other polar liquid. Thus, the transition from a first phase to a second phase can provide controlled release, on-demand delivery of the compound.

LIQUID CRYSTAL COMPOSITION AND METHOD OF USE

FIELD OF INVENTION

The present invention relates to the field of liquid crystal compositions and their methods of use. More particularly, the invention relates to liquid crystal
5 compositions that, upon change of phase, release at least one chemical species.

In one particular aspect of the present invention the liquid crystal compositions are suitable for use as a carrier for pharmaceuticals, particularly pharmaceutical actives for treatment of humans or animals.

In another aspect the present invention is suitable for industrial use such
10 as in electronic devices.

It will be convenient to hereinafter describe the invention in relation to therapeutic treatment, however it should be appreciated that the present invention is not limited solely to that use but could also be used for agrochemical treatment or other applications. Furthermore the present invention will be described with
15 reference to release of pharmaceutical actives but the skilled person will appreciate that compositions of the present invention could be used for release of non-pharmaceutical actives, particularly industrial actives.

Pharmaceutical compositions typically include a pharmaceutically active chemical and a carrier. A carrier should not interfere with, and preferably
20 enhances release of the active at a suitable rate. Liquid crystals carriers are known for use with certain pharmaceuticals.

Liquid crystals consist of two or more components that are capable, within certain concentration ranges, of exhibiting orientational long-range molecular order. They may also show short range order if positional long range order
25 disappears. Their molecular structure can entrap, and may stabilise some molecules.

BACKGROUND ART

It is to be appreciated that any discussion of documents, devices, acts or knowledge in this specification is included to explain the context of the present
30 invention. Further, the discussion throughout this specification comes about due to the realisation of the inventor and/or the identification of certain related art problems by the inventor. Moreover, any discussion of material such as documents, devices, acts or knowledge in this specification is included to explain

the context of the invention in terms of the inventor's knowledge and experience and, accordingly, any such discussion should not be taken as an admission that any of the material forms part of the prior art base or the common general knowledge in the relevant art in Australia, or elsewhere, on or before the priority
5 date of the disclosure and claims herein.

Liquid crystals generally

In aqueous surfactant and polar lipid phases, water associates with the hydrophilic head group of a surfactant molecule (to form a 'hydrophilic domain') rather than the hydrophobic tail (the 'hydrophobic domain'). When surfactant
10 molecules are placed in water they self-assemble to form geometric structures, the nature of which is dictated by the interplay between local and global constraints. For example, they may form two-dimensional lamellar structures, or hexagonal liquid crystal structures, or three-dimensional bicontinuous cubic structures. It is also known that they may convert between different phases in
15 response to various factors including changes in temperature and dilution. For example, they may convert between inverse micelles (L_2), inverse hexagonal phase (H_2), inverse cubic phase (Q_2), lamellar phase (L_α), normal cubic phase (Q_1), normal hexagonal phase (H_1) and micelles (L_1). In some instances these structures may only swell to a finite dilution enabling the dispersion of the liquid
20 crystal into particles in excess water. In the case of lamellar, hexagonal and cubic phase, these particles have been termed liposomes, hexosomes and cubosomes respectively. Structures of this type are disclosed for example in US patent 5,531, 925 (Landh *et al*) and International publication WO 2007/140510 (Monash University).

25 Many liquid crystalline systems have centred around glyceride-based lipids, monoolein (glycerol monooleate) (GMO) (Figure 1A) and monolinolein which form bicontinuous cubic structures in excess water. Many phospholipids have also been known to form liquid crystalline structures in excess water. Surfactants with a glycerate headgroup have been reported to also form liquid
30 crystalline structures in excess water.

More recently lipids with phytanyl and farnesyl hydrophobic tails have also been identified to form liquid crystalline structures in excess water.

Phytantriol (3,7,11,15-tetramethylhexadecane-1,2,3-triol) (PHYT) (Figure 1A) is a polar lipid principally known for use in cosmetics and hair-care products. For example US patent 5,834,013 (Ribier *et al* assigned to L'Oreal) describes the use of PHYT and a water soluble surface active agent acting as a stabiliser in dermatological or cosmetic products.

PHYT and GMO form similar liquid crystalline structures in water and exhibit similar phase change behaviour. In particular both form a bicontinuous cubic structure in excess water at room temperature and both form a reverse hexagonal structure in excess water at higher temperatures.

10 Liquid crystal delivery systems

Lipid-based liquid crystal systems have been shown to provide sustained release of drug molecules with a range of physicochemical properties. As described in the review by Kaasgaard and Drummond (T. Kaasgaard, C.J. Drummond, Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent, *Phys. Chem. Chem. Phys.* 8 (2006) 4957-4975), such systems are prepared using amphiphilic lipids that are able to spontaneously self-assemble into ordered liquid crystalline structures on exposure to excess water (Figure 1B). Their ability to resist further phase change to micelles or solutions, allows their application as persistent reservoirs for drug delivery applications. The structures are thermodynamically stable and comprise discrete lipidic and aqueous domains, allowing the incorporation of molecules of varying physicochemical properties.

The nanoscale internal structure of the liquid crystalline materials often includes the lamellar phase, inverse hexagonal phase (H_2) or inverse bicontinuous cubic phase (Q_2), that coexist with excess aqueous solution. Some lipid-water combinations form the Q_2 phase at lower temperatures, with a transition to the H_2 phase at higher temperatures. Perhaps the most extensively studied system that exhibits this behaviour is the GMO + water system, with increasing interest being paid to the PHYT + water system.

There has been much interest in the potential application of nanostructured lipid systems, such as the Q_2 and H_2 phases, in drug delivery due to their potential for sustained release and biocompatibility. The liquid crystalline structures formed by amphiphilic lipids such as PHYT in excess water are

thermodynamically stable. Temperature and pressure induced transitions between, for example, the L_α to H_2 phases in phospholipid-based systems have been well studied. Providing there is no chemical degradation of the lipid or other alterations in the system, transitions between phases as a result of temperature change are reversible. The use of phase transformations in these systems has been reported previously to allow administration of a low viscosity matrix by injection, which transforms into a viscous liquid crystalline phase on dilution in the body. (S. Engström, L. Lindahl, R. Wallin, J. Engblom, A study of polar lipid drug carrier systems undergoing a thermo-reversible lamellar-to-cubic phase transition, *Int. J. Pharm.* 86 (1992) 137-145; M. Malmsten, Phase transformations in self-assembly systems for drug delivery applications, *J. Disp. Sci. Tech.* 28 (2007) 63-72.)

Liquid crystal carriers for pharmaceuticals are typically formulated for injectable, topical or oral administration. Liquid crystal carriers are carefully matched to the chemical they carry to ensure the resultant formulation releases the chemical at the desired rate. This requires identification of the appropriate liquid crystal phase to provide the desired release rate for a given dose of the composition. The release of drug from lipid-based liquid crystal systems is diffusion controlled, with the generally larger aqueous channels of the bicontinuous cubic phase providing a faster release rate than the inverse hexagonal phase.

Stimuli responsive delivery systems

Stimuli responsive drug delivery systems have potential use in these situations. They also have potential use in therapeutic situations where continuous absorption of drug is not desirable (such as for toxicity reasons) by providing drug release on demand. They may also be of use where repeat injection of short acting or rapidly cleared drugs, for example peptide hormones is required, due to the potential to reduce injection frequency. The ability to effectively switch drug release on or off from an injectable depot system at will provides great control over the potential effectiveness of therapy.

Stimuli responsive drug delivery systems have not only been designed in the lipid field, but also in the polymer field for example in respect of liposomes. Functionalisation of the polymer can impart the system with sensitivity to a wide

range of stimuli including temperature, light, pH, and salt concentration to name a few. The stimulus induces a change in polymer gel structure facilitating release of drug from the polymer matrix (see for example, S.K. Ahn, R.M. Kasi, S.C. Kim, N. Sharma, Y.X. Zhou, Stimuli-responsive polymer gels, *Soft Matter* 4 (2008) 1151-1157). Polydispersity of the polymer, kinetic reproducibility, and toxicity imparted by stimuli responsive functional groups can limit the utility of these materials as responsive in vivo drug delivery systems. In most cases these systems undergo irreversible phase transition to release a drug at a locus, such as tumours.

There is a need to identify delivery systems that allow improved control over chemical release overcoming some of the limitations of existing delivery systems such as polymer-based systems. There is also a need for on-demand stimuli responsive systems for compound release.

SUMMARY OF INVENTION

An object of the present invention is to provide an improved stimuli responsive delivery system.

A further object of the present invention is to provide improved control of chemical release from a carrier.

A further object of the present invention is to alleviate at least one disadvantage associated with the related art.

It is an object of the embodiments described herein to overcome or alleviate at least one of the above noted drawbacks of related art systems or to at least provide a useful alternative to related art systems.

Method of Controlled Delivery

In a first aspect of embodiments described herein there is provided a method of controlled compound delivery from a composition comprising a liquid crystal carrier and a compound to be delivered, the method comprising the step of applying external stimulus to control transition of the liquid crystal from a first phase having a first capacity for retention of the compound to a second phase having a second capacity for retention of the compound.

Where used herein, the term 'capacity for retention' means the ability of the liquid crystalline matrix to reduce the apparent diffusion rate of the compound within the matrix to less than that in the bulk solvent coexisting with the carrier, such as excess water, bodily fluid or other polar liquid.

Thus, the transition from a first phase to a second phase can provide controlled release, on-demand delivery of the compound.

Preferably the liquid crystal carrier is lipid-based, comprising lipids that form liquid crystal structures on contact with aqueous solutions. The aqueous
5 solutions could be derived, for example, from body fluids, reaction mixtures, cell cultures or any other in vivo or in vitro source. While any suitable surfactant may be used in the liquid crystal carrier, GMO-based surfactants, phospholipids and phytantriol, and their mixtures are preferred.

Optimally the liquid crystal carrier has a nanoscale internal structure that
10 includes the lamellar phase, inverse cubic micellar phase, inverse hexagonal phase (H_2), inverse bicontinuous cubic phase (Q_2) or coexisting mixed phases thereof, that coexist with excess aqueous solution, such as excess body fluids.

The compound carried by the liquid crystal and intended for delivery may be any chemical species, but is preferably chosen from the group comprising
15 pharmaceuticals, cosmetics, agrochemicals and other industrial chemicals. In addition, a second, third and further compounds may be retained by the carrier. Each of the compounds may have the same or different retention capacities with respect to the first and second phases, or other phases of the carrier. The term 'pharmaceutical' is used herein in its broadest sense to include any molecule that
20 is bioactive for humans or other animals including, but not limited to, drugs, vitamins, proteins, peptides (eg. octreotide acetate, insulin), antibodies (eg bevacizumab, ranibizumab), hormones, radioactive species or prodrugs. The term 'cosmetics' is used herein to refer to any molecule that is used principally for its ability to enhance the appearance of tissue or protect tissue, such as
25 sunscreen. The term 'agrochemicals' is used herein in its broadest sense to include any molecule that is bioactive for plants including, but not limited to nutrients, hormones, fungicides, pesticides or growth factors that contribute to plant production. The term 'industrial chemicals' is used herein in its broadest sense to include any molecule that is not an agrochemical, cosmetic or
30 pharmaceutical but has economic value.

The external stimulus may be any source of energy suitable for converting the liquid crystal carrier from a first phase to a second phase. This may include, for example a particular wavelength of electromagnetic radiation (ie radio waves,

x-rays, microwaves, infrared rays, ultraviolet rays or visible light), magnetic field, electrical field or thermal radiation. The external stimulus may be controlled by any convenient method including manual or automated control. For example, a computer program could be used to facilitate electronic control.

- 5 Typically, when the external stimulus is removed the crystal carrier reverts from the second phase to the first phase. Alternatively a source of energy of different type, frequency, wavelength, amplitude or intensity can be used to stimulate the change from the second phase to the first phase. If the liquid crystal comprises multiple phases, one or more external stimuli may be used to trigger transformation between phases.

10 The composition may further comprise additives to alter the characteristics of the liquid crystal carrier. Typically these additives will function in one of two ways:

- 15 (1) modifying the susceptibility of the liquid crystal carrier to change phase when subjected to an external stimulus and/or
- (2) triggering phase transition in response to external stimulus, acting locally within the composition.

(1) Modifying the susceptibility of the liquid crystal carrier to change phase

- 20 In particular, the additive may either raise or alternatively lower the transition temperature depending on the application. For example, some drug delivery compositions comprising liquid crystal carriers having an inherent phase transition temperature that is very close to normal physiological temperatures of about 36° to 41°C (eg liquid crystals comprising the lipid monoelaidin). These compositions have the advantage that phase transition may be readily induced by simple means such as administration of a heat pack to the patient's body at the site of subcutaneous injection of the composition. This may work well in controlled environments such as a hospital. However in other less controlled environments, the composition may be too sensitive to physiological changes. A patient administered with a dose of the drug delivery composition may inadvertently experience raised body temperature due to factors such as exertion, fever, sauna or sunbaking, causing undesirable phase transition and drug release. Accordingly, in this situation it would be desirable to modify the phase
- 25
- 30

transition temperature to avoid physiological range, such as, $>45^{\circ}\text{C}$. Agrochemical or industrial applications may require transition temperature to be modified to occur at a quite different temperature.

Alternatively, some drug delivery compositions comprising liquid crystal carriers have an inherent phase transition temperature that is very high and there is no convenient external stimulus available to trigger phase change. An additive may be used to decrease the transition temperature to a more convenient value.

Many hydrophobic species are suitable for modification of the transition temperature. For example addition of vitamin E acetate alters the phase transition behaviour of some liquid crystal carriers, particularly those comprising PHYT. Addition of oleic acid alters the phase transition behaviour of some liquid crystal carriers, particularly those comprising GMO.

(2) Triggering phase transition

Typically this type of additive generates thermal energy to trigger the phase transition, although other forms of energy may trigger the transition. The additive may do so in response to any convenient external stimulus such as a particular wavelength of electromagnetic radiation (ie x-ray, microwave, infrared, ultraviolet or visible light), a magnetic field or heat. This type of additive is less likely to be affected by physiological or environmental changes extraneous to the application of the intended external stimulus.

Typically, when the composition comprises a pharmaceutical active, it is administered to a human or animal subject by any appropriate means. In a preferred embodiment, the composition may be injected (for example, subcutaneously, intramuscularly or intraocularly) or applied to a tissue surface (for example, topically, intranasally, intraorally, buccally or sublingually). The application of the external stimuli can thus be used to switch on or switch off the release of the pharmaceutical active from the composition to the patient. This is particularly effective when the payload of composition is injected subcutaneously and retained under the skin, or within the aqueous humor or the vitreous humor of the eye. In the case of ocular diseases such as macular degeneration and diabetic retinopathy patients can replace 3 monthly intraocular injections with a single annual intraocular injection and 3 monthly exposure to a painless external stimulus such as a laser.

In another aspect of embodiments described herein there is provided a method of controlled compound delivery from a composition comprising a lipid-based liquid crystal carrier and a first pharmaceutical, the method comprising the step of applying external stimulus to convert the liquid crystalline phase structure of the carrier to a different liquid crystalline phase structure, such as from a Q₂ structured phase having a first capacity for retention of the compound to an H₂ structured phase having a second capacity for retention of the compound.

The external stimulus for delivery of the pharmaceutical may be for example, thermal energy. Further to this the temperature of the Q₂ to H₂ transition may be suppressed for example, by addition of small amounts of vitamin E acetate if it is a PHYT + water system, or small amounts of oleic acid if it is a GMO + water system. Optimising the Q₂ to H₂ transition temperature close to body temperature (37°C) thus provides a temperature responsive liquid crystal system which uses temperature as an 'on-off' switch to control drug release.

15 Dosage Method

In a further aspect of embodiments described herein there is provided a dosage method using a composition comprising a liquid crystal carrier and a compound to be dosed, the method comprising the step of applying external stimulus to control transition of the liquid crystal from a first phase having a first capacity for retention to a second phase having a second capacity for retention wherein the second capacity is less than the first capacity and the dosage is a function of the difference between the first and second capacities.

The transition from first phase to second phase is triggered by the external stimulus and typically, when the stimulus is removed or soon thereafter, the liquid crystal carrier reverts from the second phase to the first phase. Thus dosage can be 'switched on' by applying the external stimulus or 'switched off' by removing the external stimulus. Accordingly the dosage may also be a function of the length of time period the external stimulus is applied or alternatively, the length of time the liquid crystal carrier is in the second phase.

30 With particular reference to pharmaceuticals, this provides the potential for a simplified dosage regime for a patient that can be readily controlled by a health care professional or the patient themselves by turning on and turning off an external stimulus such as a laser. Alternatively the dosage regime could be programmed

into a computer or other automated system controlling the external stimulus. The automated system may for example, turn the external stimulus on or off according to a predetermined routine, or in response to patient information such as physiological information (including body fluids composition, blood pressure or heart rate). The system is particularly well suited to frequently injected biological therapies such as peptides, proteins and antibodies, and frequently injected antipsychotic (eg. risperidone) or pain relief medications (eg. bupivacaine), where 'on demand' release of drug without re-administration is required to alleviate symptoms.

10 **System**

In yet a further aspect of embodiments described herein there is provided a system for controlled release of a compound, the system comprising a liquid crystal structure that is capable of converting from a first phase to a second phase in response to application of external stimulus, wherein the first phase has a first capacity for retention of the compound and the second phase has a second capacity for retention of the compound.

The system may be used for myriad applications. While the use of the system for drug delivery is described in detail the system may be used, for example for release of other compounds such as dyes to act as a visual marker, or charged particles to alter current flow in an electronic system. The system may also be used for remote release of chemical reagents in industrial synthesis. The system may also be used for remote release of growth factors and other chemicals in maintenance and growth of stem cells, cell culture and tissue engineering. The system may also be used as a temperature sensing device, releasing a detectable substance in response to transient temperature changes.

Composition

In yet a further aspect of embodiments described herein there is provided a composition comprising a compound and a liquid crystal carrier for controlled release of the compound, the liquid crystal carrier being capable of converting from a first phase to a second phase in response to application of external stimulus, wherein the first phase has a first capacity for retention of the compound and the second phase has a second capacity for retention of the compound.

In yet a further aspect of embodiments described herein there is provided a composition comprising;

- a compound,
- a liquid crystal carrier for controlled release of the compound, the liquid crystal carrier being capable of converting from a first phase to a second phase in response to application of external stimulus,
- an additive for modifying the behaviour of the response to external stimulus,

wherein the first phase has a first capacity for retention of the compound and the second phase has a second capacity for retention of the compound, and conversion from the first phase to the second phase releases at least some of the compound from the liquid crystal carrier.

Formulations that will work in this capacity include any liquid crystal forming lipid, in particular phytantriol, GMO, oleyl glycerate, phytanyl glycerate, glyceryl phytanoate, monoelaidin, dioleoylphosphatidylethanolamine, dioleoyl phosphatidyl choline, and mixtures thereof, in all ratios and comprising up to 99.5% w/w of the composition.

The additive typically comprises a hydrophilic, hydrophobic or amphiphilic molecule that resides in the liquid crystal structure or in the hydrating water, and its presence influences the phase transition temperature of the liquid crystal system, and is present at up to 90% w/w of the composition. The additive is typically a hydrophobic compound that resides in the lipid region of the matrix, such as vitamin E acetate. The additive may also be the drug/agent to be released from the matrix. The drug/agent to be released is present in the formulation at a level required to exert its effect when released from the matrix. The formulation may also contain water or other suitable polar liquid or solution in which the drug/agent to be released is dissolved prior to mixing with the liquid crystal forming lipid and additive, or acts to dissolve the drug or agent after mixing with the lipid and additive, and may be present at up to 99% w/w of the formulation. Hence, one formulation that would exemplify the invention would comprise 1 mg octreotide acetate dissolved in 4.5 mg water containing 0.5 mg acetic acid, mixed with 3 mg vitamin E acetate and 97 mg of phytantriol.

Other aspects and preferred forms are disclosed in the specification and/or defined in the appended claims, forming a part of the description of the invention.

In essence, embodiments of the present invention stem from the realisation that the rate of chemical release when using liquid crystal carriers can be controlled through control over the nanostructure of the liquid crystal.

Advantages provided by some or all of the embodiments of the present invention include the following:

- the ability to manipulate nanostructure to provide direct impact on chemical release;
- provision of an 'on' and/or 'off' switch for chemical release to a system;
- control of delivery of a compound at a locus, such as pharmaceuticals or cosmetics to a human or animal patient, agrochemicals to a plant or industrial chemicals to an apparatus or process;
- a new method for dosage of a chemical to a living or non-living system.

Further scope of applicability of embodiments of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure herein will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Further disclosure, objects, advantages and aspects of preferred and other embodiments of the present application may be better understood by those skilled in the relevant art by reference to the following description of embodiments taken in conjunction with the accompanying drawings, which are given by way of illustration only, and thus are not limitative of the disclosure herein, and in which:

Figure 1 illustrates the chemical structures for (i) phytantriol (PHYT), (ii) glyceryl monooleate (GMO), and (iii) vitamin E acetate (VitEA).

Figure 2A illustrates differences in release rate for glucose from Q₂ and H₂ liquid crystalline matrices at 37°C prepared from (i) GMO, or (ii) PHYT with vitamin E acetate and (iii) PHYT without vitamin E acetate (L₁ = micelles; H₁ = hexagonal; L_α = lamellar; H₂ = reversed hexagonal; L₂ = reversed micelles).

(Reproduced from K.W.Y. Lee, T.-H. Nguyen, T. Hanley, B.J. Boyd, Nanostructure of liquid crystalline matrix determines in vitro sustained release and In vivo oral absorption kinetics for hydrophilic model drugs, *Int. J. Pharm.* 365 (2009) 190-199).

5 Figure 2B is a phase diagram for partial pseudobinary PHYT + excess water - vitamin E acetate phase illustrating the temperature dependence of the (i) Q_2 , (ii) $Q_2 + H_2$, (iii) H_2 and (iv) L_2 phase regions on vitamin E acetate concentration (Reproduced from Y.D. Dong, I. Larson, T. Hanley, B.J. Boyd, Bulk and dispersed aqueous phase behaviour of phytantriol: Effect of vitamin E
10 acetate and F127 polymer on liquid crystal nanostructure, *Langmuir* 22 (2006) 9512-9518)

Figures 3A & 3B include phase diagrams assembled from small angle x-ray scattering data for matrices in excess phosphate buffered saline (PBS) with varying lipid composition, to identify optimal compositions that display the 'switch'
15 temperature in the physiological range. Specifically, Figure 3A illustrates phase behaviour observed for PHYT with increasing concentration of vitamin E acetate ((i) = $Q_2 + PBS$; (ii) = $Q_2 + H_2 + PBS$; (iii) $H_2 + PBS$). Figure 3B illustrates GMO with increasing concentration of oleic acid. Circles represent samples displaying the bicontinuous cubic phase (Q_2 , Pn3m space group), triangles represent
20 inverse hexagonal (H_2) phase, circles and triangles together represent coexisting Q_2 and H_2 phases ((i) = $Q_2 + H_2O$; (ii) = $Q_2 + H_2 + H_2O$). The boxed region in Figure 3A is the region of interest for Figure 4.

Figure 4 depicts small angle x-ray scattering profiles for matrices in excess water for the PHYT + 3% vitamin E acetate cubic phase system with increasing
25 temperature. 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 ((i) – H_2 , 39°C; (ii) $Q_2 + H_2$, 37°C; (iii) $Q_2 + H_2$, 35°C; (iv) $Q_2 + H_2$, 33°C; and (v) Q_2 (Pn3m) 31°C.)

Figure 5 illustrates the dependence of lattice parameter from small angle x-ray scattering profiles for matrices in excess water for the PHYT + 3% vitamin E
30 acetate system on increasing temperature. Closed symbols (●) represent presence of Q_2 (Pn3m) phase, open symbols (○) represent presence of H_2 phase.

Figure 6 illustrates static release profiles at constant temperature for glucose from the bicontinuous cubic (Q_2 (•)) at 30°C, and inversed hexagonal liquid crystalline matrices (H_2 (o)) at 40°C formed by PHYT + 3% vitamin E acetate, into phosphate buffered saline, plotted against square root of time. Solid lines indicate linear fits to release profiles (data are mean $\pm$ standard deviation, n=3).

Figures 7A & 7B include dynamic release profiles for glucose into phosphate buffered saline from PHYT + 3% vitamin E acetate with changing temperature, plotted against square root of time (data are mean $\pm$ standard deviation, n=3). Temperature was switched from (i) Q_2 , 30°C to (ii) H_2 , 40°C to (iii) Q_2 , 30°C at the times indicated by the dashed lines (Figure 7A) and from (i) H_2 , 40°C to (ii) Q_2 , 30°C to (iii) H_2 , 40°C (Figure 7B).

Figure 8 illustrates normalised plasma concentration profiles for ^{14}C -glucose after subcutaneous administration to rats. Plasma concentration after administration of the aqueous solution formulation is denoted by closed circles (•), PHYT alone denoted by open triangles (∇) and PHYT + 3% vitamin E acetate (switch formulation, denoted by closed triangles ($\blacktriangle$)). Dashed line indicates time at which subcutaneous temperature was 'switched' from (i) 40°C to (ii) 30°C. Data are mean $\pm$ SEM, n=3. Lines in both panels are intended as a guide to the eye only. Negative error bars (same magnitude as positive error bars) omitted for clarity.

Figure 9 is a plot of the proportion of Allura Red released against square root of time to illustrate the release mechanism according to the present invention.

Figure 10 – illustrates mean percentage of Allura Red released over time from Phytantriol + 3% vitamin E acetate liquid crystalline phases in excess water when the temperature was set to (i) 30°C from 0-24 hr (Q_2), (ii) 40°C from 24-72 hr (H_2), and (iii) 30°C from 72-140 hr (Q_2).

DETAILED DESCRIPTION

The nanoscale internal structure of liquid crystal materials for use in the present invention presents as different phases. These typically include a lamellar phase, inverse hexagonal phase (H_2) or inverse bicontinuous cubic phase (Q_2), that coexist with excess aqueous solution. Some lipid-water combinations form

the Q₂ phase at lower temperatures, with a transition to the H₂ phase at higher temperatures. The temperature of the Q₂ to H₂ transition may be suppressed by addition of small amounts of an additive, such as for example, vitamin E acetate to a PHYT + water system as seen in Figure 2B, or oleic acid to a GMO + water system.

The release of drug from lipid-based liquid crystal systems is diffusion controlled, and generally the larger aqueous channels of the bicontinuous cubic phase provide a faster release rate than the inverse hexagonal phase.

Figure 2A illustrates the release of hydrophilic drugs from a PHYT-based Q₂ structure as compared with the significantly slower release from the H₂ phase (prepared by addition of 10% vitamin E acetate to PHYT) at 37°C. This difference in release behaviour can be used between phases, using temperature as an external stimulus. Furthermore optimising the phase transition temperature to around body temperature (37°C) provides a temperature responsive liquid crystal system for which temperature could be used as an 'on-off' switch to control drug release.

Example 1

This principle of controlled release has been exemplified using a composition comprising a lipid-based liquid crystal system which was subcutaneously injected. In particular the carrier of the composition was adapted for transition between a Q₂ to H₂ phase adjusted to a transition temperature close to 37°C. Specifically, PHYT: vitamin E acetate and GMO:OA ratios to obtain a 'switch' temperature for the Q₂ to H₂ phase transition of approximately 37°C.

The nanostructure of the system was confirmed using crossed polarising microscopy and small angle x-ray scattering. With a view to the ultimate application of the technology to the release of hydrophilic peptides such as octreotide, a simple model hydrophilic drug, glucose was chosen, for in vitro release and in vivo absorption studies. Glucose was chosen principally for ease of analytical determination, and because the trends in release rates are transferable to larger hydrophilic compounds (although the release of octreotide is significantly slower than that of glucose). In vitro release of radiolabelled ¹⁴C-glucose from the systems was determined before and after heating and cooling to control the nanostructure. The temperature responsiveness was investigated in

vivo in rats by determination of drug absorption before and after application of a heat pack and a cool pack at the site of subcutaneous injection to control the subcutaneous temperature and hence the liquid crystalline matrix and drug release and absorption rates.

5 ***Transition Temperature Optimization by Crossed Polarised Light Microscopy (CPLM) and Small Angle X-ray Scattering (SAXS)***

CPLM

CPLM was used to identify the approximate range of compositions at which the Q₂ to H₂ phase transition occurred at 37°C for the PHYT + vitamin E acetate and GMO + oleic acid mixtures to guide, and to compare with, subsequent SAXS investigations. Transitions were identified by a change from the non-birefringent stiff appearance of the Q₂ phase to the birefringent 'fan-like' texture of the H₂ phase at the interface with excess aqueous solution. Lipid mixtures were prepared containing between 2.5 and 4% oleic acid or vitamin E acetate in GMO or PHYT respectively, by melting the two lipids at 60°C and vortex mixing in a glass vial. A drop of lipid mixture was placed between two coverslips and the sample flooded with phosphate buffered saline (PBS, pH 7.4) from the side by capillary action. The resulting liquid crystalline textures at the interface between lipid and PBS were viewed using a Zeiss Axiolab E microscope fitted with a Canon Powershot digital camera and a Linkam HFS 91 heating stage and a TP-93 temperature programmer. A magnification of x15 was used to observe the phases as they were heated from room temperature to 50°C at a rate of 1°C per minute. The images were recorded at temperatures above and below the observed transition temperature.

25 **SAXS**

Samples of LC phases were prepared by weighing the appropriate amounts of the lipid/s and PBS into glass vials, such that the aqueous component comprised 50% (w/w) of the total (thus ensuring an excess of aqueous solution in equilibrium with the fully swollen liquid crystal phase). The lipidic and aqueous materials were then thoroughly mixed with a three-times repeat cycle of heating to approximately 70°C, vortex mixing and centrifugation at 2800 x g. The liquid crystal phases were then equilibrated on a tube roller at 37°C for 48 hr.

Samples were packed into a custom built stainless steel paste cell with path length of ca. 2 mm defined by a Teflon spacer sealed between Kapton tape windows. The paste cells were then inserted into a thermostated metal heating block controlled by a Peltier system accurate to $\pm 0.1^\circ\text{C}$. The samples were
5 inserted into the beamline of 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 $\text{CuK}\alpha$ radiation wavelength 1.54 Å. The SAXS camera was fitted with a Hi-star 2D detector (effective pixel size 100 μm).
10 The sample to detector distance was chosen to be 650 mm, which provided a q-range of 0.008 to 0.32 $\text{\AA}^{-1}$.

Samples were equilibrated for 15 min (previously determined as an appropriate equilibration period for these samples in this apparatus with incremental temperature changes), and scattering patterns were collected over
15 15 min under vacuum to minimise air scatter. Temperature was then advanced by 2°C and the samples further equilibrated for 15 min before acquisition as above, and so on until data was obtained from 31°C to 45°C in 2°C intervals. Scattering files were background subtracted and normalized to sample transmission then integrated using Bruker AXS software v4.1.18 to the one-
20 dimensional scattering function $I(q)$, where q is the length of the scattering vector, defined by $q = (4\pi/\lambda)\sin \theta$, λ being the wavelength and 2θ the scattering angle. Peak positions in $I(q)$ vs q plots were indexed to Miller Indices against known space groups to identify phase structure. The mean lattice parameter, a , was calculated from the interplanar distance, d using the appropriate scattering law for
25 the phase structure. For cubic phases, $a = d(h^2+k^2+l^2)^{1/2}$, while for H_2 phase, $a = 4d/3(h^2+k^2)^{1/2}$, where h , k and l are the Miller indices for the particular structure present.

In vitro release studies

In vitro drug release studies were performed in triplicate based on the
30 United States Pharmacopoeial (USP) rotating-basket dissolution method using an Erweka DT6 Dissolution Tester. Temperature in the dissolution bath was controlled to $\pm 0.5^\circ\text{C}$ by an Erweka recirculating thermostatted heater unit. The release medium consisted of 500 mL PBS at pH 7.4, chosen as a model for

physiological interstitial space with a view to these systems being administered as a subcutaneous injection.

The carrier containing glucose was prepared as described above under the heading 'SAXS sample preparation and analysis', except that the PBS contained 0.25 μCi of radiolabelled glucose, and comprised 30% v/v of the total matrix (just below the excess water boundary composition to prevent excess glucose solution being present on immersion of the sample into the release medium). Approximately 400 mg of glucose-containing liquid crystal phase was accurately weighed into glass micro-beakers with an approximate volume of 400 μL and diameter of 9 mm, ensuring a well-defined reproducible surface area (50.2 mm^2) for glucose release. Knowing the accurate weight of matrix in the beaker allowed calculation of the 100% release value in disintegrations per minute. These filled mini-beakers were placed inside the dissolution basket and set to rotate at 100 rpm. The release studies were continued for one week at 30°C or 40°C for the 'static' release studies, or switched between the two temperatures at predetermined time points for the 'dynamic' release studies. At the sampling time points, a 200 μL sample was removed from the release medium and replaced by 200 μL PBS, and the sample mixed with 2 mL of scintillation cocktail in a 6 mL scintillation vial for scintillation counting on a Tri-Carb 2800TR, Perkin Elmer Liquid Scintillation Analyser.

In vitro release data analysis

Release data were converted to percentage of ^{14}C -glucose released against time. As previous studies have shown that liquid crystalline systems display diffusion-controlled release, data were plotted as drug release versus square root of time ($t^{1/2}$). Diffusion coefficients were calculated from the slope of the % released vs $t^{1/2}$ plots using the previously derived expression for diffusion-controlled release from a single sided matrix

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

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.

Proof of principle in vivo subcutaneous absorption animal study

Animal Procedures

The subcutaneous absorption study was conducted using rats (male, Sprague Dawley, 250-330 g). Animals were anaesthetised prior to surgery. The
5 anaesthetised rats were placed on a heated surface maintained at 37°C and were cannulated via the carotid artery. For the duration of the experiment, the rats were laid on their left sides on the 37°C surface.

A thermometer accurate to $\pm 0.2^\circ\text{C}$, was inserted into a subcutaneous pocket on the right side of the midline of the back immediately adjacent to the
10 injection site, to monitor subcutaneous temperature prior to dosing. The homeostatic subcutaneous temperature was approximately 35°C. The subcutaneous temperature was increased to 40°C by the application of a small heat pack immediately prior to injection and after 4 hours, decreased to 30°C by the application of an ice pack onto the skin and maintained to within $\pm 0.5^\circ\text{C}$ for
15 the duration of the experiment. At the conclusion of the experiments, animals were sacrificed via infusion of 0.5 mL Lethobarb into the carotid cannula.

Blood samples (0.2 mL) were obtained via the indwelling cannula and cannulas were kept patent by flushing with a small (0.2 mL) volume of 1 IU/mL heparin in saline. Blood samples were placed immediately into a tube containing
20 10 IU of heparin and plasma was separated by centrifugation for 7 min at 2800 x g. Plasma (100 μL) was removed, to which 1 mL of Starscint scintillation cocktail was added. The sample was then vortex mixed before analysis by liquid scintillation counting.

Formulation preparation and administration

25 Direct dosing of liquid crystalline systems is impossible due to their inherently high viscosity, thus a low viscosity lamellar phase (L_α) liquid crystal precursor system was prepared that forms Q_2 or H_2 on exposure to excess solution, such as PBS or interstitial fluid. The precursor systems were prepared in the same manner as described above for the in vitro release samples, except that the
30 lesser amount of aqueous solution was included. It was found that the maximum amount of water that was able to be incorporated into the liquid crystal was 12% (w/w) before its viscosity prevented injection through a 27G needle. Thus, the aqueous component in which ^{14}C -glucose was incorporated comprised 12%

(w/w). The aqueous component contained a target dose of approximately 700 mg/mL glucose. The target dose in terms of radioactivity was 1.35 μ Ci.

Rats were administered one of four formulations: (i) subcutaneous (SC) administration of a glucose solution in PBS as a rapidly absorbed control
5 formulation, (ii) SC administration of a cubic phase precursor in which phytantriol alone was used as the lipid component, and was not anticipated to change phase structure with changes in subcutaneous temperature, (iii) SC administration of a precursor in which the lipid component comprised phytantriol with 3% vitamin E acetate, which was expected to change phase structure from H₂ to Q₂ when the
10 subcutaneous temperature was decreased from 40°C to 30°C, and (iv) an intravenously administered (I.V.) glucose solution. The subcutaneous formulations all contained a nominal dose of 67 mg/kg glucose, corresponding to an approximate dose of 300 mg of liquid crystal precursor formulation or 300 μ L ¹⁴C-glucose solution, via subcutaneous injection. For I.V. studies, the rats were
15 administered ¹⁴C-glucose solution (containing a nominal dose of 50 mg/kg glucose in 200 μ L saline) by injection into the tail vein. All studies were performed in triplicate. The liquid crystal precursor formulations were followed by a subcutaneous injection of 1 mL saline at the injection site in order to minimise any effect of the liquid crystal imbibing water from surrounding tissue while forming
20 the equilibrium liquid crystal structure. To determine the administered dose, residual formulation in the syringe was determined by rinsing the syringe with saline for the studies using glucose solution, or 95% ethanol for the liquid crystal precursor formulations and subsequent scintillation counting.

Pharmacokinetic Analysis

25 The data obtained as disintegrations per minute (DPM) were converted to plasma concentration using the known activity of the glucose used in this study. Plasma concentrations (μ g/mL) were normalised to a dose of 50 mg/kg. Pharmacokinetic parameters, total area under the plasma concentration-time curve (AUC), peak plasma concentration (C_{max}) and time to reach peak plasma
30 concentration (t_{max}), were calculated for all SC administrations. In order to allow more thorough interrogation of data obtained after SC dosing studies, I.V. dosing studies were also conducted (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 following equation:

$$F\% = \frac{(AUC_{sc}) \times (Dose_{lv})}{(AUC_{lv}) \times (Dose_{sc})} \times 100\%$$

Statistics

- 5 At all points in the manuscript, P values were calculated using the Student T-test, and values >0.05 were considered to be not significant.

Optimization of phase behaviour

Initial composition scans using CPLM

- 10 CPLM was used in the first instance to determine the approximate range of composition required for a Q₂ + excess PBS → H₂ + excess PBS (from hereon simply referred to as Q₂ or H₂) transition temperature in physiological range (approximately 37°C). The transition temperatures for the two lipid systems (PHYT + vitamin E acetate (PHYT + VitEA) and glyceryl monooleate + oleic acid (GMO + OA)) in excess PBS were determined.

- 15 Textures observed for the Q₂ and H₂ phases were essentially identical to those previously reported in a number of publications, for examples of typical Q₂ and H₂ textures (see J. Barauskas, T. Landh, Phase behaviour of the phytantriol/water system, Langmuir 19 (2003) 9562-9565).

- For the PHYT + VitEA system, previous studies have shown that
20 compositions containing approximately 3-4% vitamin E acetate would have a transition temperature in physiological range (Figure 2B). With increasing concentration of Vitamin E acetate in phytantriol (3.1%, 3.3%, 3.5%, and 3.8% (w/w)) the transition temperatures decreased from 36.8°C to 34.4°C to 31.4°C to 30.5°C respectively, confirming that the samples were in the approximate
25 required composition range, as well as in close agreement with the previously reported transition temperature data. For GMO, increasing the concentration of oleic acid (3, 5 and 7% (w/w)) resulted in decreasing transition temperatures from 35°C to 30°C to 28°C respectively. Hence approximately 3% oleic acid in GMO was identified as an appropriate composition to further study at greater resolution
30 using SAXS.

Phase boundary determination using SAXS

SAXS scattering profiles were used to determine phase identity at defined compositions with changes in temperature. These were particularly useful because SAXS is able to readily distinguish multi-phase regions (likely to complicate release data) which is difficult using CPLM. Results from SAXS investigations on compositions covering those estimated as the optimal ranges from CPLM are illustrated as phase diagrams in Figure 3.

Figure 3A illustrates the phase behaviour of the PHYT + VitEA mixtures in excess PBS, and shows that a concentration of vitamin E acetate very close to 3% (w/w) is required in order that at 30°C the sample exhibits only Q₂ phase, and at 40°C exhibits only H₂, with the Q₂+H₂ transition region falling between the two. Again the width of the transition region agreed well with previous work in Figure 2B despite that work using a much lower composition resolution. Figure 4 illustrates the change in SAXS profile with increasing temperature for the PHYT + 3% vitamin E acetate system, revealing the conversion from the Q₂ phase (with reflections spaced at $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, and $\sqrt{6}$ corresponding to the Pn3m spacegroup in agreement with previous findings) to the H₂ phase (reflections at 1, $\sqrt{3}$ and $\sqrt{4}$). The lattice parameter is an important determinant of the dimensions of the aqueous domains of the liquid crystal and hence its dependence on temperature is of interest for this study. The changes in lattice parameter for the PHYT + 3% vitamin E acetate system are shown in Figure 5 and reveal an approximate lattice parameter of 62 Å for the Q₂ (Pn3m) phase, and approximately 48 Å for the H₂ phase. Although there is a steady decrease in lattice parameter with increasing temperature the change is of the order of <2 Å across the 30°C to 40°C temperature range, indicating that changes in lattice dimensions are unlikely to significantly influence drug release rates across this temperature range. The lattice parameters for the Q₂ and H₂ phases were also independent of vitamin E acetate concentration within the range 2.5 – 3.5% w/w in phytantriol, meaning that from the perspective of the impact of phase structure on drug release, subtle changes in composition are unlikely to influence drug release rates.

Figure 3B shows the equivalent results for the GMO + OA system, and reveals that the Q₂ + H₂ region is very wide – in fact no pure H₂ regions were identified for the samples even at 46°C, highlighting the extra benefit in

characterizing such systems using multiple techniques, and in particular SAXS. The Q₂ phase for GMO + OA also possessed the Pn3m spacegroup with lattice parameter 83.3 Å at 31°C, while at 45°C, the lattice parameter of the coexisting Q₂ phase had reduced to 80.3 Å, and the lattice parameter for the coexisting H₂ phase was 58.6 Å. In consideration of the wider aims of the study to investigate the effect of switching between the Q₂ and H₂ phase structures, and the subsequent influence on drug release and absorption, the GMO + OA system was not further investigated, and subsequent studies utilized phytantriol containing 3% vitamin E acetate.

10 In vitro drug release

Drug release at static temperature (30°C and 40°C)

In order to first establish the relative drug release rates from each individual phase structure (and subsequently derived diffusion coefficient for the model hydrophilic drug in the PHYT + 3% VitEA matrix), release studies were conducted where the temperature was maintained at either 30°C or 40°C for the duration of the experiment. The data in Figure 6 illustrate the amount of model drug released against square root of time to interrogate the release mechanism. The linear profiles for % released against $t^{1/2}$ confirm that release was under diffusion control. The release from the Q₂ phase at 30°C was faster than from the H₂ phase at 40°C (phase structures determined from SAXS in 3.1.2 above), consistent with the previously reported results in Figure 2A, with systems designed to possess the Q₂ and H₂ phase structures at 37°C. This result is reflected in the calculated diffusion coefficients in Table 1 of approximately 75 and 12 x 10⁻⁸ cm².sec⁻¹ for glucose in the Q₂ and H₂ phases respectively. The diffusion coefficients for glucose in the Q₂ phase agreed with the literature value (reproduced in Table 1), and the approximately 7-fold difference between the diffusion coefficients from the Q₂ and H₂ phases was also observed, although the value for the H₂ phase in this study was higher than that from the previously reported value (P = 0.0039).

30 **Table 1** – Diffusion coefficient (x 10⁻⁸ cm².sec⁻¹) for glucose in the Q₂ (inverse bicontinuous cubic phase) and H₂ (inverse hexagonal phase) liquid crystalline matrices comprising phytantriol + 3% vitamin E acetate with varying temperature, calculated from release data (mean ± s.d., n = 3).

Temp (°C)	Phase	Static (no switch)	30 →40 →30 switch	40 →30 →40 switch
30	Q ₂	75 ± 4	71 ± 2	-
40	H ₂	12 ± 1	0.4 ± 0.5	7.2 ± 0.3
30	Q ₂	-	160 ± 10	188 ± 3
40	H ₂	-	-	0.04 ± 0.04
37 [†] (lit)	Q ₂	74 ± 3	-	-
37 ^{†*} (lit)	H ₂	7 ± 1	-	-

† From K.W.Y. Lee, T.-H. Nguyen, T. Hanley, B.J. Boyd, Nanostructure of liquid crystalline matrix determines in vitro sustained release and in vivo oral absorption kinetics for hydrophilic model drugs, Int. J. Pharm. 365 (2009) 190-199.

*Contained 10% vitamin E acetate to suppress phase transition to below 25°C.

Drug Release under dynamic temperature/phase conditions ('Switching')

Experiments were conducted to investigate the effect of changing phase structure on drug release *in situ*. This was accomplished by 'switching' the temperature between 30°C and 40°C to stimulate the phase changes between the Q₂ and H₂ phases respectively. The reversibility of the transition was also tested by switching back to the starting temperature to change back to the original phase structure, and hence original drug release rate. The release data on switching from 30°C→40°C→30°C, and hence from Q₂→H₂→Q₂ are illustrated in Figure 7, Panel A. There is a clear suppression of drug release on switching the phase structure from Q₂ to H₂ as would be expected from the static data in Figure 6, and on switching the phase structure back to Q₂, the system appeared to return to close to the original release rate. The linear profile when plotted against $t^{1/2}$ again demonstrates that the diffusion of drug within the matrix is controlling the release rate, and that the diffusion behaviour is reversible on changing back to the original Q₂ phase. The calculated diffusion coefficients did not support a strictly reversible transition between structures – the diffusion coefficient in the Q₂ phase whilst initially maintained at 30°C agreed well with the 'static' diffusion coefficient for the Q₂ phase, however, after switching back from the H₂ phase to the Q₂ phase, the diffusion coefficient had increased significantly to over 160 x 10⁻¹⁰

⁸ cm².sec⁻¹. The profiles were linear indicating that an effectively equilibrium structure had been achieved in both cases. The diffusion coefficient for glucose in the H₂ phase in the interim period at 40°C was also significantly different to that in the static experiment, i.e. less than 1 x 10⁻⁸ cm².sec⁻¹.

- 5 For the experiment in which the starting phase was the H₂ structure at 40°C and a 40°C→30°C→40°C (i.e. H₂→Q₂→H₂) set of transitions was undertaken, an analogous set of conclusions was apparent; visually the release rates appear to be reversible and approximately in line with what would be anticipated from the static experiments and anticipated phase structures. The
- 10 diffusion coefficient in Table 1 for glucose in the initial H₂ matrix agreed well with that from the static experiment (P=0.47). However, on comparison of the diffusion coefficients after switching phase structures, significant differences between the diffusion coefficients for glucose in the two H₂ regions is apparent, with the second H₂ region imparting a much lower diffusion rate on the drug than
- 15 that at the start of the experiment (0.04 vs. 7.2 x 10⁻⁸ cm².sec⁻¹). The Q₂ phase also imparted a faster diffusion rate on the glucose than in the static experiment at 30°C, but was similar to that of the Q₂ phase after reversion back to Q₂ in the 30°C→40°C→30°C switching experiment.

In vivo Absorption Studies

- 20 The plasma profiles obtained after subcutaneous injection of liquid crystal precursor formulations containing glucose, and an aqueous glucose solution as control formulation, are illustrated in Figure 8. Broadly speaking the profiles were different for the different formulation types, despite all containing glucose as the model drug at the same concentration. The rapid absorption of glucose from the
- 25 aqueous solution over the first hour, then decay of glucose on elimination and/or distribution to other tissues was evident. Administration of the precursor consisting of phytantriol alone, which was expected to form the Q₂ phase in vivo, showed a longer t_{max} (P<0.01). Due to the variability in plasma concentrations for the aqueous solution formulation, the C_{max} between the solution formulation and
- 30 the Q₂ precursor were not statistically different (P=0.1581), even though the C_{max} appears lower visually for the Q₂ precursor. There was no apparent influence of the subcutaneous temperature change from 40°C to 30°C at 240 min after

administration on the plasma profiles for either the aqueous solution or the Q₂ precursor.

In the case of the phytantriol + vitamin E acetate 'switch' formulation, the initial phase structure anticipated to form on administration at 40°C was the very slow release H₂ phase. Drug absorption in the first 4 hr was reduced to a large degree after administration of this formulation compared to the Q₂ formulation (but not entirely halted), although it should be noted that Figure 8 is a semi-log plot, and the C_{max} was significantly lower compared to the Q₂ formulation (P<0.001). After switching the subcutaneous temperature down to 30°C, there was a definitive increase in absorption such that not only was the glucose elimination arrested, but the plasma concentrations actually continued to rise after the 300 min sample. This finding strongly supports our hypothesis that these systems have application as on-demand stimuli responsive delivery systems.

The pharmacokinetic parameters derived from the profiles in Figure 8 are detailed in Table 2. Of particular interest is the decreasing trend in C_{max} for glucose when administered in the PHYT + Vitamin E Acetate formulation vs PHYT alone vs. Solution vehicles. The flatter profile after administration of the 'switch' formulation also resulted in a longer average T_{max}, consistent with slower release from the H₂ phase in Figure 6.

Table 2 - Pharmacokinetic parameters for ¹⁴C-glucose after subcutaneous and intravenous administration in rats (dose normalised to 50 mg/kg). Values are given as mean ± SEM (n = 3). Abbreviations: Phyt = phytantriol alone; Phyt + VitEA = Phytantriol + 3% Vitamin E acetate; Solution = ¹⁴C-glucose solution in phosphate buffered saline; IV = Intravenous infusion of ¹⁴C-glucose in phosphate buffered saline. AUC = area under plasma concentration versus time curve; C_{max} = average peak plasma concentration; t_{max} = average time to reach peak plasma concentration; F% = absolute oral bioavailability.

	Solution	PHYT	PHYT + VitEA	IV
AUC	14800 ±			32100 ±
(µg/mL·min)	2200 ^a	6598 ± 460 ^a	18300 ± 1800 ^a	2100
C_{max} (µg/mL)	89 ± 13	66.2 ± 1.6	37.8 ± 2.6	-
t_{max} (min)	54.7 ± 4.7	101 ± 11	173 ± 93	-
F%	46.1 ± 6.9	20.5 ± 1.4	57.1 ± 5.7	-

^aAUC truncated to 480 min.

Discussion

- The systematic increases in $t_{\max}$ after administration of model drug from aqueous solution to Q₂ to H₂ precursor systems illustrates the concept of slow in vitro release from the Q₂ phase, and slower from the H₂ phase, leading to differences in absorption rate and appearance in plasma. This trend was observed in the initial stages after administration and was also observed after the oral administration of Q₂ and H₂ precursors.
- The initial absorption of glucose after administration of the switching formulation at 40°C was lower than for the Q₂ phase, but was still at a measureable level. In eventual application, if this phase is to constitute the 'off' position in terms of drug release, it is desirable for the release rate of drug, and subsequent absorption to be very low in the initial stages after administration before the switching stimulus is applied. The likely explanation for the appearance of drug in plasma at the early times from the H₂ phase, not anticipated from the very slow release seen in Figure 6, is that the surface area of the formulation is much greater after administration through the needle into the subcutaneous space. The overall geometry of the injection is difficult to control absolutely under these administration requirements. On the other hand, switching the temperature to 30°C and stimulating an increased rate of absorption (by virtue of Q₂ formation) facilitated a plasma profile that was very flat (mean plasma concentrations were 33.5 ± 1.8 mg/mL at all points after the 20 min time point). This presents an alternate opportunity to use these systems to provide long term maintenance of drug concentration within a tight range for drugs with

longer half lives, in contract to the original 'on/off' concept to provide a spike in plasma concentration on demand which is likely to be most effective for drugs with short half-lives. This approach might be useful in mimicking a steady state infusion but with a single injection.

5 The use of SAXS to optimize the compositions to provide reversible structural changes between Q₂ and H₂ phase succeeded, agreed with previous SAXS studies on these systems, and also agreed well with the dynamic in vitro release data in Figure 7. The reversibility of the release behaviour in Figure 7 at first glance was notable, however, conversion of release data to diffusion
10 coefficients in Table 1 revealed some systematic differences in diffusion of the model drug in these systems. Closer inspection of the data reveal that diffusion of glucose in Q₂ phase before undergoing a switch to H₂ phase, in all three cases (the static 30°C, the first stage of the 30°C → 40°C → 30°C switch study and the referenced study at 37°C) provided a diffusion coefficient of 70-75 x 10⁻⁸ cm².sec⁻¹.
15 However, diffusion in the Q₂ phase after it has been generated by reducing the temperature from the H₂ phase was approximately 160 and 187 x 10⁻⁸ cm².sec⁻¹. Similarly, for systems in the H₂ phase that had not undergone a phase change to Q₂ phase, the diffusion of glucose was >5 x 10⁻⁸ cm².sec⁻¹, while it was <1 x 10⁻⁸ cm².sec⁻¹ for the two that had undergone a prior phase change to the H₂ phase.
20 The reason for these apparently systematic differences in diffusion are unclear at this stage – the reversibility of phase structure for these systems has been previously demonstrated, and it is extremely unlikely that chemical degradation is responsible as it did not matter in what order the temperature switching were conducted. Although the differences do not change the overall observation of
25 diffusion in Q₂ phase being significantly faster than H₂, they may impact on the reproducibility of these systems and hence more studies to investigate this phenomenon using SAXS during release studies are planned.

Controlling the release of molecules 'on-demand' from self assembled structures in aqueous environments also has potential for application in the food,
30 cosmetic, chemical manufacture and consumer products fields to name a few. Protection and release of nutraceuticals such as vitamins has become a strong parallel field of research to drug delivery, with food applications of liquid crystalline structures being reviewed recently although truly stimuli responsive

'functional foods' are not yet a reality. In the cosmetic field, light induced activation of UV absorbing compounds may be a route to long lasting sunscreen products, while selective release of compounds from liquid crystals under particular conditions in reactors could provide the next generation of approaches to processes such as emulsion polymerization.

This study has provided an initial proof of concept for the use of liquid crystalline matrices as on-demand stimuli responsive systems for hydrophilic drugs. Further studies will utilise drug molecules of particular interest such as short acting hormones like octreotide acetate. Reversibility *in vivo* will also be investigated, as will the broader application of these systems designed to respond to other stimuli.

Example 2

In vitro release experiments at different temperatures were also conducted using a different model hydrophilic drug 'Allura Red' using the same matrix as that in Example 1 (i.e. phytantriol containing 3% w/w vitamin E acetate). These experiments again show the ability to 'switch' drug release between different rates by changing the liquid crystal nanostructure between two different liquid crystalline structures.

Drug release at static temperature (30°C and 40°C)

In order to first establish the relative drug release rates for Allura Red from each individual phase structure in the PHYT + 3% VitEA matrix, release studies were conducted where the temperature was maintained at either 30°C or 40°C for the duration of the experiment. The data in Figure 9 illustrate the amount of Allura Red released against square root of time to interrogate the release mechanism. The linear profiles for % released against $t^{1/2}$ confirm that release was under diffusion control. The release from the Q₂ phase at 30°C was faster than from the H₂ phase at 40°C.

Experiments were conducted to investigate the effect of changing phase structure on drug release *in situ*. This was accomplished by 'switching' the temperature between 30°C and 40°C to stimulate the phase changes between the Q₂ and H₂ phases respectively. The reversibility of the transition was also tested by switching back to the starting temperature to change back to the original phase structure, and hence original drug release rate. The release data on

switching from 30°C→40°C→30°C, and hence from Q₂→H₂→Q₂ are illustrated in Figure 10. There is a clear suppression of drug release on switching the phase structure from Q₂ to H₂ as would be expected from the static data in Figure 9, and on switching the phase structure back to Q₂, the system appeared to return to
5 close to the original release rate. The linear profile when plotted against $t^{1/2}$ again demonstrates that the diffusion of drug within the matrix is controlling the release rate, and that the diffusion behaviour is reversible on changing back to the original Q₂ phase.

While this invention has been described in connection with specific
10 embodiments thereof, it will be understood that it is capable of further modification(s). This application is intended to cover any variations uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be
15 applied to the essential features hereinbefore set forth.

As the present invention may be embodied in several forms without departing from the spirit of the essential characteristics of the invention, it should be understood that the above described embodiments are not to limit the present invention unless otherwise specified, but rather should be construed broadly
20 within the spirit and scope of the invention as defined in the appended claims. The described embodiments are to be considered in all respects as illustrative only and not restrictive.

Various modifications and equivalent arrangements are intended to be included within the spirit and scope of the invention and appended claims.
25 Therefore, the specific embodiments are to be understood to be illustrative of the many ways in which the principles of the present invention may be practiced. In the following claims, means-plus-function clauses are intended to cover structures as performing the defined function and not only structural equivalents, but also equivalent structures. For example, although a nail and a screw may not be
30 structural equivalents in that a nail employs a cylindrical surface to secure wooden parts together, whereas a screw employs a helical surface to secure wooden parts together, in the environment of fastening wooden parts, a nail and a screw are equivalent structures.

"Comprises/comprising" and "includes/including" when used in this specification is taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof. Thus, unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', 'includes', 'including' and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

CLAIMS

1. A method of controlled compound delivery from a composition comprising a liquid crystal carrier and a compound to be delivered, the method comprising
5 the step of applying external stimulus to control transition of the liquid crystal from a first phase having a first capacity for retention of the compound to a second phase having a second capacity for retention of the compound.
2. A method according to claim 1 wherein the first phase of the liquid crystal
10 carrier has a nanoscale internal structure chosen from the group comprising lamellar phase, inverse micellar cubic phase, inverse hexagonal phase (H_2), inverse bicontinuous cubic phase (Q_2) or coexisting mixed phases thereof.
3. A method of controlled compound delivery from a composition comprising
15 a lipid-based liquid crystal carrier and a first pharmaceutical, the method comprising the step of applying external stimulus to convert the carrier between a Q_2 structured phase having a first capacity for retention of the compound and a H_2 structured phase having a second capacity for retention of the compound.
- 20 4. A method according to any one of claims 1 to 3 wherein the liquid crystal includes a surfactant chosen from the group consisting of GMO-based surfactants, phospholipids, phytantriol and mixtures thereof.
5. A method according to any one of claims 1 to 3 wherein the external
25 stimulus is chosen from the group comprising electromagnetic radiation, magnetic fields, electrical fields, thermal radiation or combinations thereof.
6. A method according to any one of claims 1 to 3 wherein the phase change
30 is affected by an additive in the composition, the additive being chosen from the group comprising (1) phase change susceptibility modifiers, (2) phase transition triggers, or combinations of (1) and (2).

7. A method according to claim 6 wherein the additive affects the inherent phase transition temperature of the liquid crystal carrier.
8. A dosage method using a composition comprising a liquid crystal carrier
5 and a compound to be dosed, the method comprising the step of applying external stimulus to control transition of the liquid crystal from a first phase having a first capacity for retention to a second phase having a second capacity for retention wherein the second concentration is less than the first concentration and the dosage is a function of the difference between the first and second capacity
10 for retention.
9. A dosage method according to claim 8 wherein the compound is chosen from the group comprising pharmaceuticals, cosmetics, agrochemicals and industrial chemicals.
15
10. A dosage method according to claim 8 or claim 9 wherein the composition is administered to a subject by and administration route chosen from subcutaneous, intramuscular, intraocular, intranasal, intraoral, buccal, sublingual, topical, or combinations thereof.
20
11. A dosage method according to any one of claims 8 to 10 wherein the external stimulus is chosen from the group comprising electromagnetic radiation, magnetic fields, electrical fields, thermal radiation or combinations thereof.
- 25 12. A system for controlled release of a compound, the system comprising a liquid crystal structure that is capable of converting from a first phase to a second phase in response to application of external stimulus, wherein the first phase has a first capacity for retention of the compound and the second phase has a second capacity for retention of the compound.
30
13. A system for controlled release of a compound according to claim 12, wherein upon removal of the external stimulus, the liquid crystal structure reverts from the second phase to the first phase.

14. A system according to claim 12 or claim 13 wherein the compound is chosen from the group comprising pharmaceuticals, cosmetics, agrochemicals and industrial chemicals.

5 15. A composition comprising;

- a compound,
- a liquid crystal carrier for controlled release of the compound, the liquid crystal carrier being capable of converting from a first phase to a second phase in response to application of external stimulus,
- 10 - an additive for modifying the behaviour of the response to external stimulus,

wherein the first phase has a first capacity for retention of the compound and the second phase has a second capacity for retention of the compound, and conversion from the first phase to the second phase releases at least some of the
15 compound from the liquid crystal carrier.

16. A composition according to claim 15 comprising a liquid crystal forming lipid chosen from the group comprising phytantriol, GMO, oleyl glycerate, phytanyl glycerate, glyceryl phytanoate, monoelaidin, dioleoylphosphatidylethanolamine,
20 dioleoyl phosphatidyl choline, and mixtures thereof, comprising up to 99.5% w/w of the composition.

17. A composition according to claim 15 which further comprises an additive chosen from the group comprising (1) phase change susceptibility modifiers, (2)
25 phase change triggers, and combinations of (1) and (2).

18. A composition according to claim 17 wherein the additive is chosen from the group comprising hydrophilic, hydrophobic or amphiphilic molecules, comprising up to 90% w/w of the composition.

30

19. A composition according to claim 17 wherein the additive is vitamin E acetate.

20. A method according to claim 1 and substantially as herein described with reference to the examples.
21. A dosage method according to claim 8 and substantially as herein
5 described with reference to the examples.
22. A system according to claim 12 and substantially as herein described with reference to the examples.
- 10 23. A composition according to claim 15 and substantially as herein described with reference to the examples.

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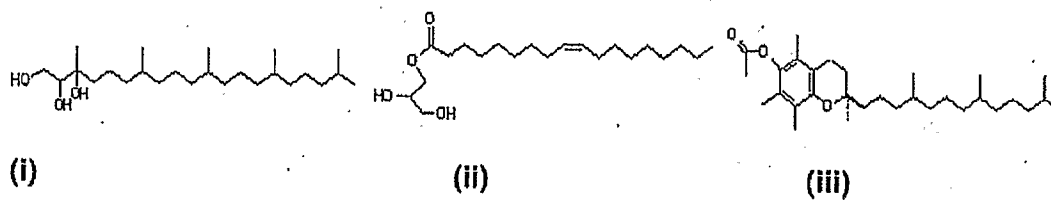


FIGURE 1A

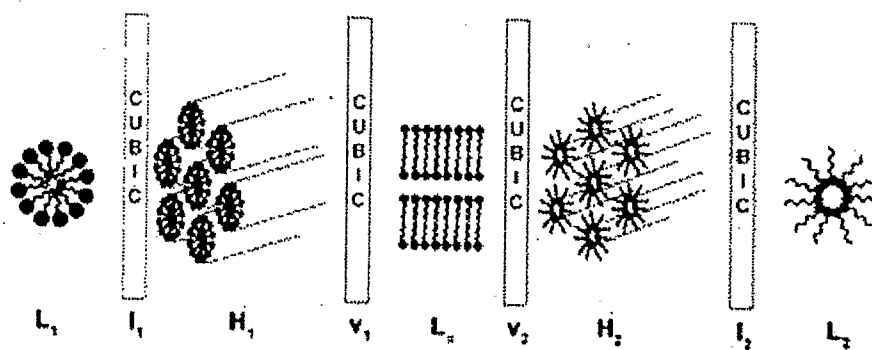


FIGURE 1B

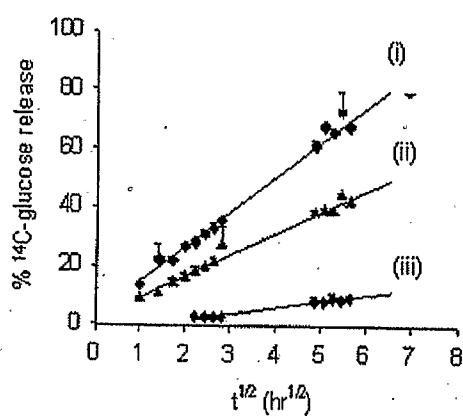


FIGURE 2A

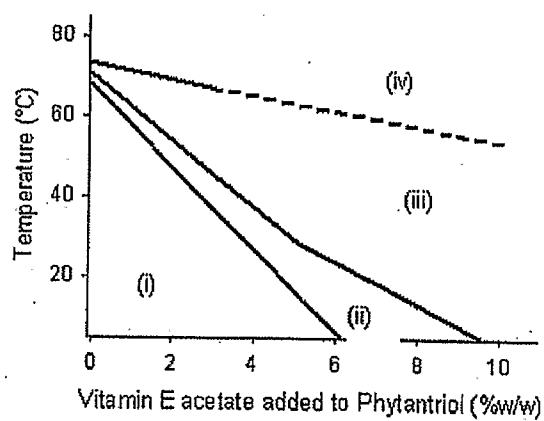


FIGURE 2B

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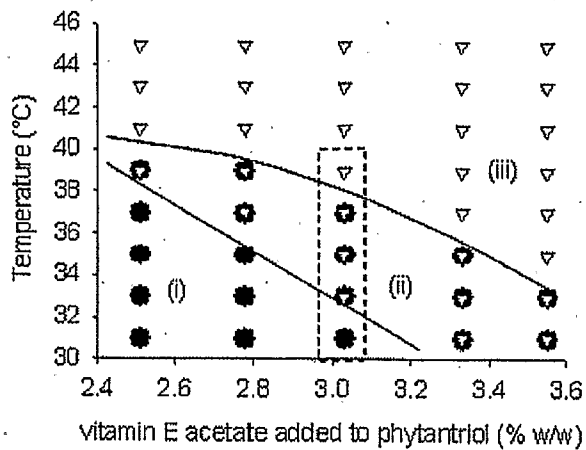


FIGURE 3A

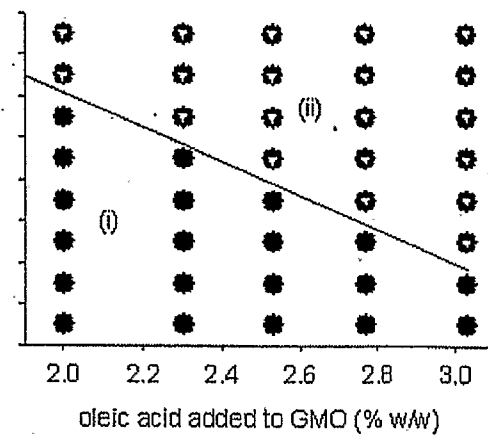


FIGURE 3B

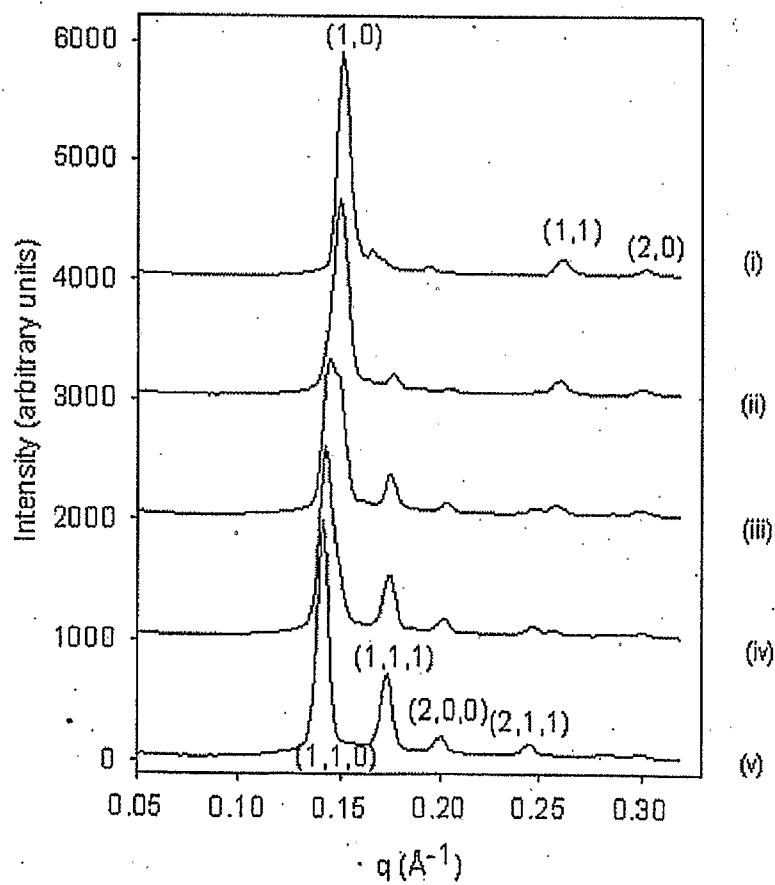


FIGURE 4

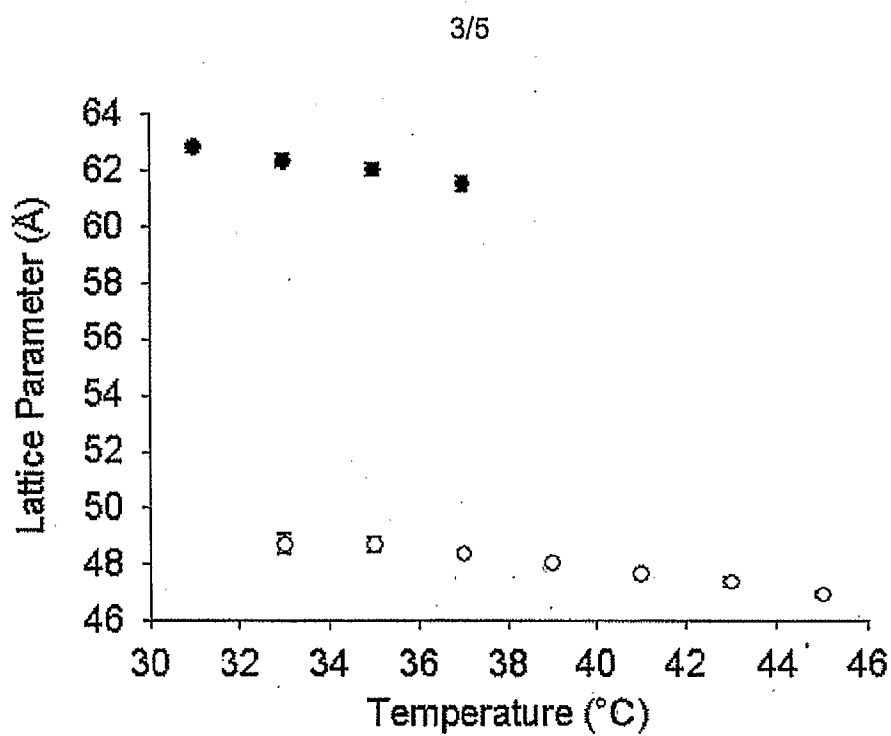


FIGURE 5

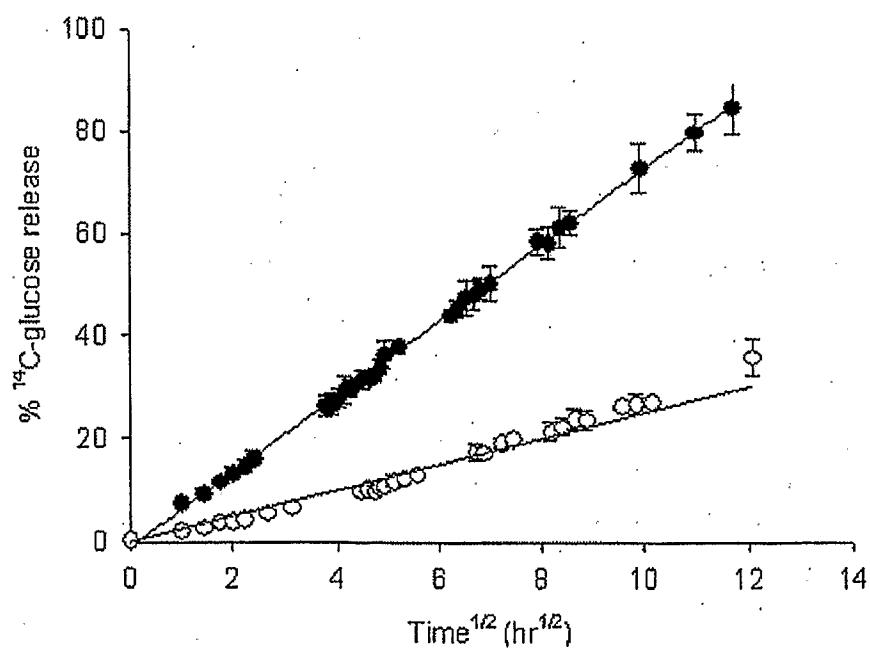


FIGURE 6

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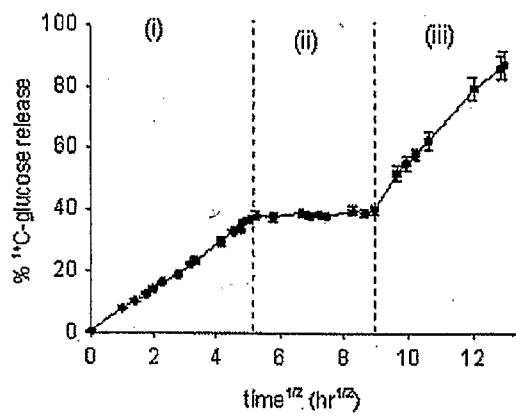


FIGURE 7A

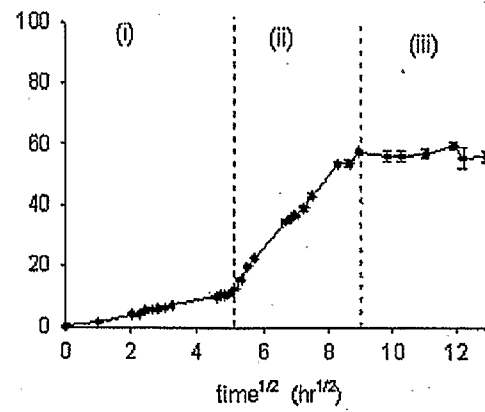


FIGURE 7B

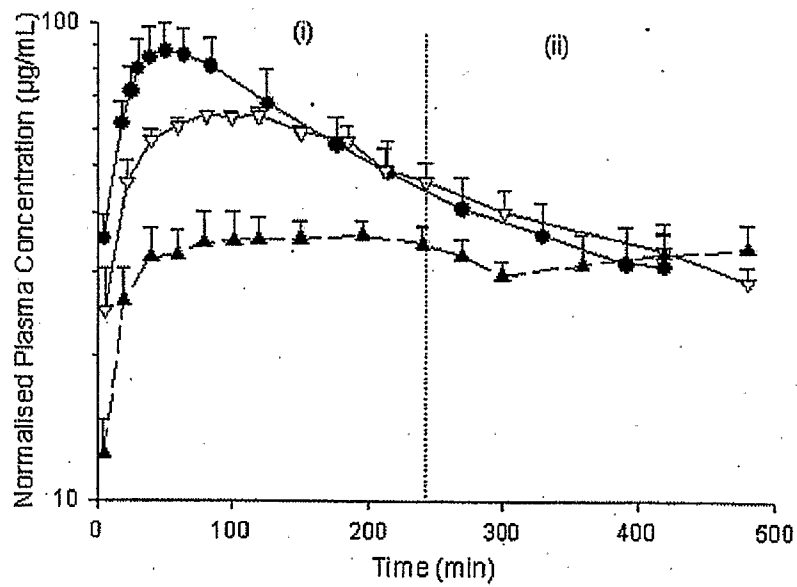


FIGURE 8

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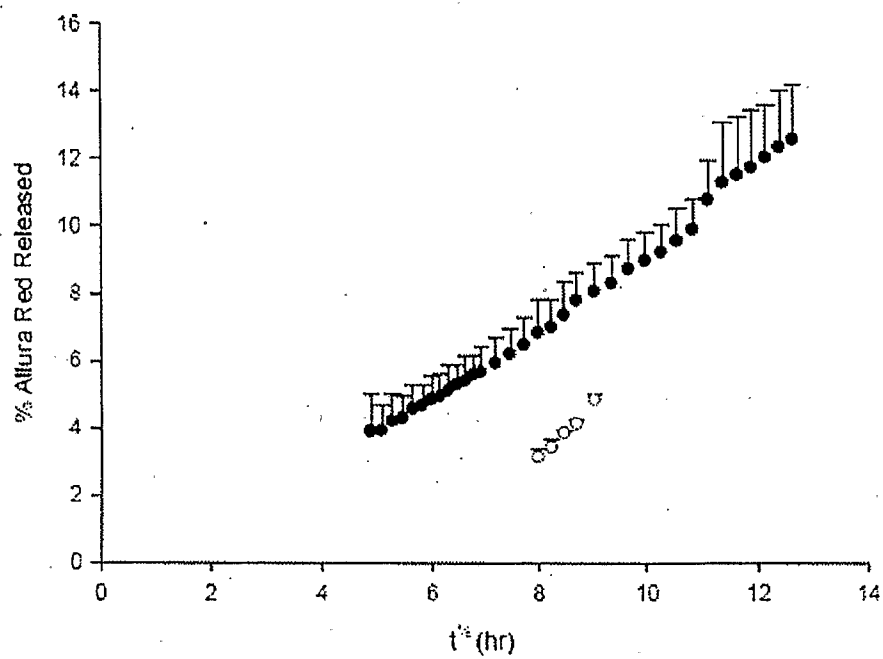


FIGURE 9

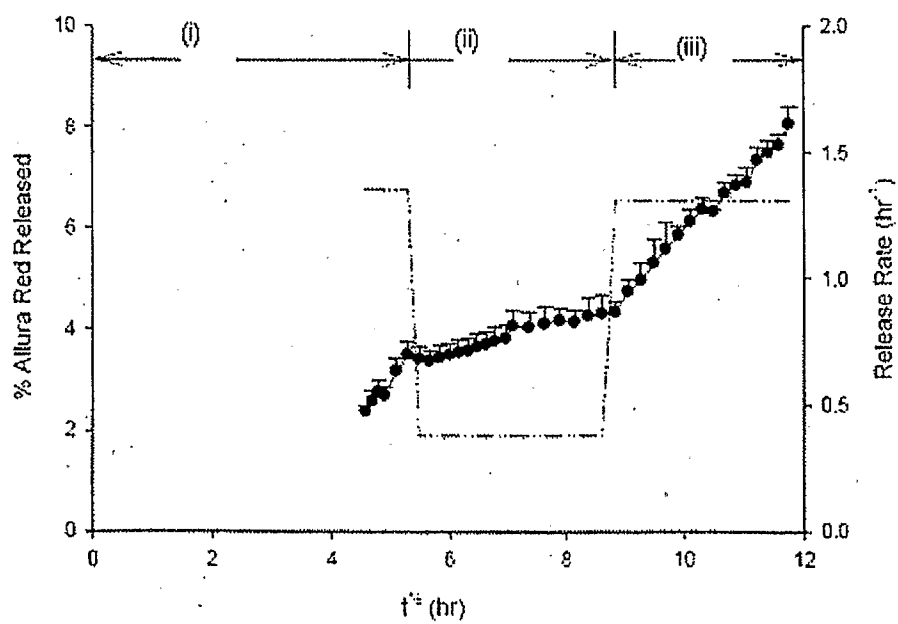


FIGURE 10

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2009/001418

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C09K 19/02 (2006.01) **A61K 9/52** (2006.01) **C09K 19/52** (2006.01)
A61K 8/02 (2006.01) **C09K 19/06** (2006.01) **C09K 19/54** (2006.01)
A61K 8/67 (2006.01) **C09K 19/42** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 WPI, EPODOC: liquid_crystal+, carrier+, deliver+, release+, change+, transition+, transform+, drug+, IPC A61K

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2000/000033 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 6 January 2000 Pages 19, 24, 39, 43, 44, 47, 53, Figure 10	1, 2, 4-19
X	US 4,513,034 A (SPARER et. al.) 23 April 1985 Col. 2-4	1, 5, 8-14
X	WO 1998/030206 A1 (GS DEVELOPMENT AB) 16 July 1998 Pages 4, 5, 7	1, 2, 4, 6, 8-12, 14-18
X	MARRINK, SIEWERT-JAN et al. Molecular Dynamics Simulation of Spontaneous Membrane Fusion during a Cubic-Hexagonal Phase Transition. Biophysical Journal, 2002, Vol. 83(5), pages 2386-2392 Introduction; Figure 6, Discussion	3

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

 Date of the actual completion of the international search
 08 December 2009/17 December 2009

Date of mailing of the international search report

22 DEC 2009
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2009/001418

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **20-23**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The claims do not comply with Rule 6.2(a) because they rely on references to the description and examples.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2009/001418

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,537,575 B1 (FIRESTONE et al.) 25 March 2003 Whole document	1-19
A	WO 2003/000236 A1 (LYOTROPIC THERAPEUTICS INC) 03 January 2003 Whole document	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2009/001418

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	0000033	AU	47030/99	AU	55936/98	EP	0958380
		US	6358523	US	2002102297	WO	9824936
US	4513034	NONE					
WO	9830206	AU	55832/98	CA	2277377	EP	0954287
		SE	9700061	US	6660278		
US	6537575	NONE					
WO	03000236	CA	2451432	EP	1408932	US	6991809
		US	2003022242				
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							