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Liquid crystal nanoparticles for commercial drug delivery

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Liquid crystals are an intermediate state of matter that exists between conventional solids and liquids. They are vital to the existence of life as several critical components in living organisms such as cell wall and biochemical fluids are liquid crystalline in nature. Drug delivery based on liquid crystals is a vast field of research. In recent years there has been a huge leap in interest into using liquid crystals, particularly lyotropic liquid crystals, as nanoparticles (cubosomes and hexosomes) for drug delivery applications. Such nanoparticle based drug delivery promise efficient, controlled and target selective release of drugs. This paper reviews the concepts and techniques involved in lyotropic liquid crystal based drug delivery. The influence of physical properties of liquid crystals on the drug carrier design and efficiency, key aspects of the methods used to identify, characterize and analyze lyotropic nanoparticles and the feasibility of production of nanoparticles for their widespread usage are discussed. The study suggests that liquid crystal based nanoparticles have the potential to revolutionize drug delivery industry, however a reliable method for production of nanoparticles on a large scale needs to be explored further.

Keywords: Lyotropic liquid crystals, drug delivery, nanoparticles

1. Introduction

Liquid crystals (LCs) are a unique state of condensed matter shown by some organic compounds. The conventional crystalline phase of matter shows a strictly ordered lattice arrangement that preserves a three dimensional positional and orientational order of the constituents, whereas, the liquid phase does possesses neither of these type of order. The

liquid crystal phases are intermediate to solids and liquids: they possess fluidity like liquids and one and two dimensional orientational and positional order similar to crystals. The average orientation direction of the liquid crystal molecules is known as the director, **n** and the orientational order is quantified by a parameter, S, which is given by $S = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle$. Here, the angled brackets indicate an average over the ensemble and θ is the angle that represents the deviation of the individual molecules from the average orientation direction. There are mainly two types of liquid crystals – thermotropics and lyotropics. Thermotropics exhibit liquid crystallinity as a function of temperature and lyotropics exhibit liquid crystallinity as a function of concentration [Figure 1]. Thermotropic liquid crystals are often associated with liquid crystal display (LCD) screens and for decades they have dominated liquid crystal research for this reason. The focus of this review article is lyotropic liquid crystals (LLC) as they are increasingly relevant to the drug delivery industry. Lyotropics are widely used for drug delivery as they provide in-vitro performance with good drug solubility in injection solvent so that various bioactive molecules can be dissolved in aqueous or oil phase to protect from oxidation and hydrolysis. Also, lyotropics are chemically stable and do not hydrolytically degrade by enzymes before reaching the target and show good absorption by human tissues.

The paper is divided as follows. The first part introduces lyotropic liquid crystals and general concepts involved in LLC based drug delivery. This is followed by a discussion of encapsulated LLC nanoparticles for drug delivery, their production and characterization and potential problems and possible future directions.

1.1 Lyotropic liquid crystals

Lyotropic liquid crystals form in the presence of a solvent. The solute molecules that form these liquid crystal phases are amphiphilic in nature having distinct polar and nonpolar units in the molecular structures. The solutes could be ionic, non-ionic or cationic [Figure 2]. The solvents can be either polar or non-polar depending upon the solute molecule chosen, however the most commonly studied solvent is water, which is a polar solvent. When the amphiphilic molecules are dissolved in water, the hydrophilic polar head and the hydrophobic carbon chain tail self-assemble into spherical aggregates called micelles. In a micelle, the hydrophilic heads of each amphiphilic molecule are exposed to the surrounding water, and the hydrophobic tails are protected inside the spherical shell created by the head groups [Figure 3]. Micelles are essentially an amphiphilic monolayer system and each of the aggregates is distributed randomly in the solvent creating an isotropic micellar solution. As more amphiphiles are added into the solution, the micelles self-assemble into long range ordered assemblies, producing multiple different lyotropic liquid crystal phases. There are mainly three types of lyotropic liquid crystal phases that are relevant. They are the (1) hexagonal (2) cubic and (3) lamellar phases [Figure 3], in the order of increasing amphiphile concentration.

The hexagonal phase is denoted as a H_1 phase and is one of the most commonly occurring lyotropic mesophases. In this phase, a monolayer of amphiphilic molecules is arranged into two dimensional cylindrical micellar aggregates of indefinite length. The columnar aggregates then assemble into a long-range hexagonal lattice. The rod-like micellar arrangement shows a diameter that is 1.3 to 2 times the alkyl chain length with a typical inter-micellar separation of 8-50 Å. Other than the hexagonal phase, there are various intermediate cubic mesophases, denoted as V_1 or I_1 formed by micelles. These include micellar cubic phase (V_1) and a series of bicontinuous cubic phases (I_1) with defined

continuous water channels [1]. The cubic mesophases are of interest in the creation of cubosomes, as the water channels have extremely high surface area (up to 400m^2/g) available for the loading of drugs [2]. A higher amphiphile concentration promotes the formation of a lamellar mesophase, denoted as L_α. The L_α phase has a bilayer structure in which amphiphiles arrange into alternating sheets of amphiphiles and water with the hydrophilic head groups protecting the hydrophobic tails within the bilayer.

In certain amphiphile and solvent binary systems, as the concentration of the amphiphile is increased, liquid crystal phases beyond the lamellar L_α phase begin to form. These phases differ to those formed in lower amphiphile concentrations (H_1, V_1 and L_α) and are known as 'inverse topology' mesophases. The difference between 'normal topology' also called Type 1 mesophases (H_1, V_1 and L_α) and 'inverse topology' (Type 2 mesophases) is that the direction in which the amphiphiles face with respect to the solvent channel is reversed. This leads to a reversal of roles of the solution constituents when compared to the regular topological mesophases. The inverse topology phases are labelled similarly to their normal topology counterparts; however the subscript is changed to represent the new type of mesophase. Reverse cubic phases is labelled as V_2, the reverse hexagonal phase as H_2 and reverse micellar cubic phase as I_2 etc. A more detailed discussion on the inverse topology mesophases is provided later.

Figure 4 shows the phase diagram of a commercial food grade emulsifier made of unsaturated monoglycerides, Dimodan U/J, in water [3]. At human body temperature, 37 ^oC, the mixture is in the lamellar phase when water content is low, and at the same temperature, the mixture is in the cubic phase when water content is higher. The figure shows the phase diagram with and without glucose added to the binary mixture.

The phase transitions in lyotropic liquid crystals are driven by a structural parameter called the packing parameter. Consider an amphiphile molecule as shown in Figure 5. If a is the surface area of the headgroup, v is the volume of the core, l is the length of the amphiphile molecule (~ length of the flexible chain, l_c) and R_1 and R_2 are the local radii of curvature, then the packing parameter can be written as

$$\frac{v}{al} = \left[1 - \frac{l}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \frac{l^2}{3R_1R_2}\right] \qquad (1)$$

For a particular liquid crystal phase, the packing parameter can be calculated from its structure. The calculation involves a direct substitution of the curvature radii into the eqn 1. For example for the hexagonal phase, $R_1 \sim l_c$, $R_2 = \infty$, hence the packing parameter is

$$\frac{v}{al} = \lim_{R_1 \sim l_c, R_2 \to \infty}\left[1 - \frac{l}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \frac{l^2}{3R_1R_2}\right] = \frac{1}{2} \qquad (2)$$

1.2 Lyotropic liquid crystals for drug delivery

In general, the principle of using lyotropic liquid crystals for drug delivery is to dissolve the compounds in the liquid crystal matrix which then carries the drugs to the pathological target. The amphiphilic surfactants that form the lyotropic liquid crystals provide fair solubility to either hydrophobic or hydrophilic drugs. The solubility or the amount of drug that is loaded can be manipulated by the addition of an enhancer. In-vivo, the carrier system can block the contact of drug with enzymes and hence protect the drugs against biochemical reactions. [4-8] The procedure of such a drug delivery comprises of two main stages: firstly preparation of the drug and liquid crystal matrix and secondly dissolving the drug in the liquid crystal matrix. Some of the most widely investigated molecules for

LLC drug delivery are monoglycerides, ethylene oxide amphiphiles, [9,10] glycolipids, [11,12] phosphatidylethanolamine amphiphiles, urea amphiphiles, [13] phytantriol, [14, 15] etc. If the drug is hydrophobic it is firstly mixed with surfactants before dissolving the aqueous components into the mixture, whereas if the drug is hydrophilic they are dissolved in the aqueous phase first and then mixed with the surfactants. Either way, the mixture is centrifuged to remove excess water. The mixture is then placed in tube roller or left alone for several days until it reaches equilibrium. There are two ways in which drugs are delivered to a target – through a sustained release or burst release. Several methods are used to evaluate the amount of drug release depending on the nature of the drugs. For example, if the drug is pre-marked by a radioactive material, then scintillation counting can be conducted; if drugs absorb ultraviolet light, UV spectroscopy is performed to identify the absorption.

1.2.1 Mechanism of sustained drug release

Hexagonal and cubic liquid crystal matrices are usually employed to perform a sustained drug release. The location where the drugs are loaded in a liquid crystal phase differs for different phases. However, overall hydrophilic drugs are loaded to the hydrophilic head groups, and hydrophobic drugs are loaded to the hydrophobic tails [Figure 6].

The characteristic of sustained release of drugs from the lyotropic mesophases is related to the high viscosity of the mesophases. If the drug is diffusion controlled, the matrix does not change its properties throughout the release process when and the matrix is very large compared to the drug, the release process obey Fick's law [16]. This law in one dimension can be written as

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (3)$$

where C is the concentration of the drug as a function of the position x and time t. D is the diffusion coefficient and is given by $D = \frac{k_B T}{6\pi\eta a}$. Here, T is the temperature, a is the hydrodynamic radius of the diffusing drug molecule and η is the viscosity of the matrix. Solving equation 3 gives the concentration of the drug as a function of position x and time t, which is then used to calculate the total amount of drug released per unit surface area Q. When different boundary conditions and initial conditions are applied, the solution to equation 3 may differ but generally, the total amount of released drug per unit surface area should be positively related to time t. Also, the Q should be positively related to the diffusion coefficient and is therefore negatively related to viscosity. Here is an example to demonstrate the relation between the amount of drug released per unit surface area Q and viscosity η. Assuming the drug is located in region 0 < x < h with uniform initial concentration C_0 and a perfect sink is set at x = 0, the total amount of released drugs per unit surface area Q is given by

$$Q = hC_0\left[1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2}\exp\left(\frac{-D(2m+1)^2\pi^2 t}{4h^2}\right)\right] \quad (4)$$

Using the approximation h→∞, the total amount of drug released per unit area Q can be roughly approximated by the following equation [17]

$$Q = \left(\frac{2C_0\sqrt{t}}{\sqrt{\pi}}\right)\sqrt{D} = \left(\frac{2C_0}{\pi}\right)\sqrt{\left(\frac{k_B T t}{6a}\right)}\sqrt{\frac{1}{\eta}} \quad (5)$$

Equation 5 shows that the amount of drug released is proportional to the square root of reciprocal viscosity. Figure 7 shows a plot of time dependence of the amount of drug released per square meter obtained from eqn. 5.

1.2.2 The mechanism of burst drug release

One of the typical systems used for burst release is particulate dispersions such as cubosomes and hexasomes. More details on cubosomes and hexasomes are provided later. The term burst release relates to the 'active' induction of the release of drug cargo. There are a number of ways including using light, heat, magnetic field and radiofrequency to provoke the active release of nanoparticle cargos. The particulate dispersions provide burst release as they have high surface area and the total amount of drug released, M, is related to the surface area A, by $M = Q \times A$. Substituting for Q from equation 5 gives

$$M = \left(\frac{2C_0}{\pi}\right)\sqrt{\left(\frac{k_B T t}{6a}\right)}\sqrt{\frac{1}{\eta}} \times A \quad (6) .$$

In equation 6, the total amount of drug released, M is directly proportional to the surface area, A and inversely proportional to square root of viscosity, η. Unlike bulk release where viscosity, η plays a significant role in the total amount of drug released, with cubosomes the surface area is so large that the term A is the predominant term controlling the release. Below in Table 1 is an example to show the prominence of the surface area term in the total amount of drug released in particulate dispersions. This compares the release from a bulk cubic phase and the release from cubosomes having the same volume [18]. In this study the matrix was made of Myverol and Lutrol F127 and Diazepam and somatostatin were used as trial drugs. The study shows that the effective surface area of cubosome is 20,000 times larger than that of bulk cubic phase matrix, which has equivalent total volume but only one exposed surface.

Table 1: Comparison between the release behaviour from bulk phase against the release from particulate dispersion. (a): Release of diazepam and somatostain from bulk cubic phase. (b): release of diazepam and somatostain from cubosome (cubic particulate dispersion). Diagonal corner to corner diameter of monodisperse cubic particles was 200

nm. In both bulk phase experiment and cubosome experiment, ratio of matrix volume against release medium was the same. [18]

	Release from bulk phase		**Release from cubosomes**	
	Diazepam	**Somatostatin**	**Diazepam**	**Somatostatin**
Effective surface area (cm^2)	0.785	1	1.7×10^5	7.8×10^4
Released drug amount Q ($mg/min^{1/2}$)	51.2	500	1.1×10^7	3.9×10^7

1.2.3 The role of physical properties

Liquid crystals are soft materials which implies that the formation and stability of mesophases are sensitive to external perturbations such as temperature, pressure, pH etc. This section discusses the influence of some of these parameters on lyotropic liquid crystal mesophases and hence on their ability to deliver drugs.

Raising the pH of the amphiphilic mixture could increase the degree of ionization of the negatively charged head groups thereby increasing the repulsion between them. As a result of stronger repulsion, the optimal surface area a increases and the packing parameter decreases. The change of packing parameter could lead to phase transitions. This sensitivity of the lyotropic liquid crystals to the pH could be made use of for drug release with changing pH value acting as a switch to control the release of drugs from the carrier. Figure 8 shows an example where different pH values within the human body causes phase transitions and affects the drugs delivery. Negrini et al [19,20] tested various liquid crystal matrices in different pH conditions and their release behaviour. Each matrix was made of monolinolein + drug + linoleic acid + excess water and was in a cubic phase in pH 7 (small intestine) and hexagonal in pH 2 (stomach). The release rate was found to be slower when it was in an acidic (pH 2) environment than that in a neutral one (pH 7).

Similar pH-responsive transitions can be used to protect drugs from leaking well before reaching the target. [21-23].

Another important parameter that control the phase transition in LLCs and hence their drug delivery is temperature [24-27]. Fonga et al [24] investigated the temperature sensitivity of the phytantriol matrix with glucose loading. The temperature was changed artificially from 40ºC to 30ºC and back to 40ºC. At 40ºC, the matrix was found to be in the reversed hexagonal phase H_2 with low release rate and when the temperature was changed to 30^0C, the matrix was in a bicontinuous cubic phase with faster release. A schematic representation of the dependence of the release process on temperature is shown in Figure 9.

Other than pH and temperature there are other important physical parameters that affect the LLC drug delivery including for example chemical compounds such as drugs, enhancer, water, etc. When a drug is loaded into a matrix, it becomes a part of the system. Therefore, the properties of the drug may affect the overall behaviour of the system. The drug itself has several parameters that could affect the release behaviour, such as the solubility, viscosity and size. [28, 29] The drug loaded to the matrix resides in the hydrophobic regions or the hydrophilic regions depending on whether the drug is hydrophobic or hydrophilic. If the drug is located in the hydrophilic portion, then the volume of that portion alters. If the drug is located in the hydrophobic portion, then the surface area changes. Either way, the packing parameter changes giving rise to phase transitions.

1.3 Encapsulated structures for drug delivery

There are several methods to deliver drugs and the number of new methods developed has seen exponential growth in the last decade. The methods in which drugs can be delivered range from topical application through the skin (transdermal), injection based applications (intramuscular, intravenous, subcutaneous), and application via mucosal cavities (transmucosal). However, the emphasis remains on the least invasive and most potent applications. Due to the ease of application and its non-invasive nature, oral delivery remains the ideal method for drug delivery in the majority of cases, however the issues of circulation times, bioavailability and system toxicity still remain to be solved. Prior to the encapsulation of drugs to prolong their lifespan within the body, non-invasive drug delivery relied primarily on gelatine pills or pressed tablets. These methods provided direct delivery of the active ingredient, limiting the efficacy of any administered drug significantly if it could not withstand the harsh environment of the digestive system.

The first breakthroughs made in encapsulated delivery date back to the mid-20th century, during which the encapsulation of pharmaceuticals on a microscopic scale was established via methods such as Wurster process. These early techniques utilised both physical and chemical methods of micro-encapsulation, covering the active ingredients with a biocompatible coating to increase their stability and circulation time [30]. Further research led to the usage of polymer-based formulations for sustained release drug delivery, an area in which the use of liposomal particles for the delivery of lipid and water-soluble pharmaceuticals was also recognised [31].

1.4 Lipid nanoparticles - Liposomes

Liposomes are considered as the first pharmaceutically viable nanoparticle to be applied as far back as the early 1980's [32,33]. They have been widely recognised for the myriad

of properties they possess that are rarely found in conventional drug delivery, such as improved bioavailability, increased loading capabilities of pharmaceuticals, and the possible modifications that can be made to the particle's features both biophysically and chemically [34, 35]. Liposomes, while considered as one of the simplest lipid based aggregates, differ from micelles in their internal structure. While micelles only contain a single layer of amphiphilic molecules, liposomes are constructed of a spherical bilayer of amphiphiles, the same as that found in the lamellar L_α mesophase. The aggregate can be described as having a single lamellar bilayer, creating a contained sphere of aqueous solution inside the liposome, which is shielded from outside contact to create nanoparticles that vary in size from 50-1000 nm [36]. This aqueous centre is where a variety of molecules may be loaded for delivery, including DNA, proteins and pharmaceuticals [37,38].

Historically, liposomes have been heralded as an extremely valuable method for drug delivery, with their usage in targeted delivery proving successful in reducing system toxicity of powerful disease fighting drugs. [39] Some pharmaceuticals, while effective against their target, can cause high levels of toxicity to the body that is detrimental to a patient's health. Such an example in which liposomal delivery has proved effective is the delivery of doxorubicin hydrochloride, in which liposomes loaded with doxorubicin hydrochloride and stabilized with polyethylene glycol (PEG) drastically reduced the toxicity to the heart's fascia when compared to the traditional administering of the drug [40] These advantages have been used in multiple applications of drug delivery, such as anticancer and antifungal drug delivery, displaying the viability of lipid nanoparticles even in the infancy of this area of study [41,42].

Despite their advantages, early liposome delivery systems suffered from an issue that any extraneous particle introduced to the human body must overcome, the defence mechanisms designed to counter unrecognised substances [43]. To address this, the use of the polymer polyethylene glycol (PEG) was added in solution to create a densely hydrated exterior. The coating sterically stabilises the liposome and aids in protecting it from interactions by the immune system, which can cause degradation of the particle either electrostatically or hydrophobically via cell breakdown and plasma interaction [44]. This use of polymers would prove to be a huge leap forward in increasing the lifespan of liposomes and at a later stage a crucial aspect of the creation and stabilisation of liquid crystal nanoparticle dispersions [45, 46]. Other modifications have also been made such as ligand-targeted liposomes, theranostic liposomes etc, to allow numerous types of drug delivery. Figure 10 shows a cross sectional view of the main types of liposomes: a conventional liposome that is able to deliver a variety of hydrophilic, hydrophobic and amphiphilic molecules, a PEGylated liposome that has been treated with polyethylene glycol to improve steric stability, a ligand-targeted liposome that is able to deliver a wide variety of site specific molecules and a theranostic liposome which is both for therapeutic and diagnostic purposes. [36]

While liposomes have proved useful for many important applications, there are still difficulties that remain unaddressed. Due to the width and curvature of the lipid bilayer, large hydrophobic molecules are often difficult to imbed within the nanoparticle shell, and cannot be held in the aqueous centre [47]. As many new anticancer drugs are hydrophobic and highly cytotoxic, a targeted and stable delivery system similar to liposomes is needed to ensure it can be delivered to the correct location without degradation or premature release. Another problem is the rate at which liposomes release

their cargo once the lipid membrane is broken. While improvements in circulation time have been observed using PEGylation, the time taken for a liposome to release the payload once the bilayer has been compromised is short and categorised as a burst release [48]. This is due to liposome's simple structure that once pierced, is subject to degradation and releases its aqueous centre and contents immediately.

A final issue to mention with liposomes is the preparation method. Due to the need to highly agitate a solution to promote the formation of liposomes, the energy requirements for the various methods are proportionately large for the amount of liposomes prepared (10-20mg) and not feasible for mass production. All methods involved begin with the freeze-drying of a solution of lipids and the selected cargo to ensure a homogenous mix in a dry film or 'lipid cake'. This is followed by reintroducing the dried lipid film to aqueous solution, which is then agitated via sonication or pressure cell extrusion. Sonication is the most common method of preparation and has the capacity to form liposomes as small as 15-50nm. It is achieved by applying high frequency sound waves of up to 20 kHz and above (ultra-sonication), which agitate the solution by creating high and low pressure areas that cause cavitation to occur. This violent creation and collapsing of cavities (bubbles) within the solution creates high temperatures and releases a huge amount of energy that disperses the large aggregates into uni-lamellar liposomes. This method however has various drawbacks. Sonication conditions are extremely difficult to replicate, meaning each resulting particle size differs from the last. Due to the high curvature and therefore low stability of the particles, they will aggregate if the temperature drops under that at which the phase transition occurs. It also requires a high amount of energy input, as homogenising the initial dispersion of large uni-lamellar vesicles (LUV) and multi-lamellar vesicles (MLV) such that only small uni-lamellar

vesicles (SUV) remain means agitating a high stability aggregate into a far more unstable state [49]. For the reasons mentioned above, a new method of delivery is needed that can replicate the benefits of the liposome while addressing its drawbacks. Drawing on the research of liposomes, cubosomes and hexosomes are a new liquid crystal nanoparticle that can potentially address these issues and provide a new and improved generation of nanoparticles.

1.5 Liquid crystal nanoparticles for drug delivery

The concept of application of nanoparticles for drug delivery was developed in early 80s [50,51]. This development followed by an extensive study of lyotropic liquid crystals led to the development liquid crystal nanoparticles for drug delivery applications. Despite the highly praised use of liposomes as the first clinically accepted nanoparticular medicine, and the advancements made in their circulation time using PEGylated liposomes and targeted delivery [47], the successive breakthrough in lipid based nanostructures has only just begun to emerge. While some nanoparticle formations have shown promise for anti-carcinogenic purposes [52], the advancement of lipid-based nanoparticles that make use of lyotropic liquid crystal mesophases, specifically cubosomes and hexosomes, has begun to flourish in the early 21st century. Possibilities such as delivering largely insoluble hydrophobic drugs, increasing bioavailability and potency of the delivered pharmaceuticals, and targeted delivery have all been reported along with lower toxicity than previous delivery methods [53].

Cubosomes and hexosomes are the first of their kind to have multilevel structural makeup, with observable complexity at the molecular, mesophasic and nanoparticular scales. Their internal structures are determined by the liquid crystal mesophase of the amphiphiles in

solution before dispersion, and are commonly of inverse topology. Due to these complex topologies inside the particles, they are able to carry greater volumes of cargo and provide sustained release [54]. Particle sizes vary from 10-1000nm and require a surfactant much like liposomes to remain dispersed and avoid aggregation. The tailored structures can be changed by substituting different amphiphiles to provide customisation on the nano-level, providing prospects towards drug personalisation [55], and the capacity for large volumes of drug cargo due to the internal structure's curvature suggests a possible increase in potency for targeted pharmaceuticals.

A number of lyotropic liquid crystal phases can be used to create nanoparticles. Both normal (type 1) and inverse (type 2) topology mesophases have been used in production of cubosomes [51, 56], while hexosomes are formed only from an inverse topological mesophases. While there are normal topological mesophases that provide viable application for drug delivery, the discussions here focus on the applicable inverse topology mesophases; the inverse bicontinuous cubic V_2 mesophases and the inverse hexagonal H_2 mesophase [57-59]. These mesophases can be dispersed to create the liquid crystal nanoparticles; cubosomes from the reverse cubic mesophases, and hexosomes from the reverse hexagonal phase.

1.5.1 The Reverse cubic phases

The first of the phases that is commonly used in the production of liquid crystal nanoparticles is the reverse cubic phases V_2. Due to their multiple complex structures, they are considered a group of mesophases rather than a solitary phase. While only three structures have been confirmed experimentally, it is theorised that there are other possible structures. However the limit of stability means that only the three variations seen in

Figure 11 have low enough curvature energy to form in observable conditions. This stability of LLCs was defined by Schwarz et al [60,61] as the curvature energy using the distribution of Gaussian curvatures. When this idea is applied to multiple possible surfaces of the reverse cubic phase the free energy densities of the gyroid (G), diamond (D) and primitive (P) structures are found to be substantially lower than other structures tested. All bicontinuous cubic structures are also unique from other mesophases as their mean curvature is zero, such that at every point they are equally convex and concave. This type of surface is known as infinitely periodic minimal surfaces, and gives the G, D and P variants their spatial groups Ia3d, Pn3m, and Im3m. The internal structure of these phases is built from a singular amphiphilic layer that is continuous throughout the structure, separating the dual adjacent water channels within the cubically symmetric arrangement.

1.5.2 The Reverse Hexagonal Phase

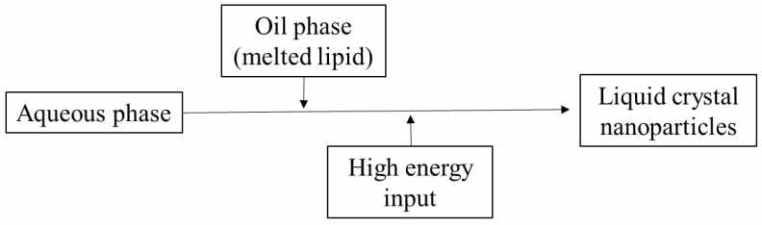
The reverse hexagonal phase H_2 is very similar to the H_1 phase and is much simpler than the reverse cubic phases in structure. Formation of this phase occurs at a higher amphiphilic concentration than the cubic phases and is formed of two dimensional micellar columns similar to H_1. While the structure of this phase is simple to understand, the hexagonal lattice is quite sensitive to the parameters of the solution when it comes to achieving a stable formulation [62]. Due to the hexagonal packing of the lattice, the reduction of empty spacing between amphiphile tails is conducive to the stabilisation of the lattice. This is because the volume of empty spacing is directly proportional to the diameter of the encompassed water channel, which itself is inversely proportional to the curvature of the water channel surface. Therefore, the reduction in diameter of the water channel and subsequent increase in curvature will lead to less empty spacing and greater

stability of the lattice. Research carried out using phospholipids by Duesing et al [63]. confirmed the large impact of stable packing of the lattice for the reversed hexagonal phase by observing that 54% of the free energy of the structure was due to geometric frustration. In comparison, just 1% of the free energy of the reversed cubic phase was attributed to geometric frustration, demonstrating the different requirements for stability between continuous and non-continuous mesophases.

1.5.3 Nanoparticle Preparation: technologies and methodologies

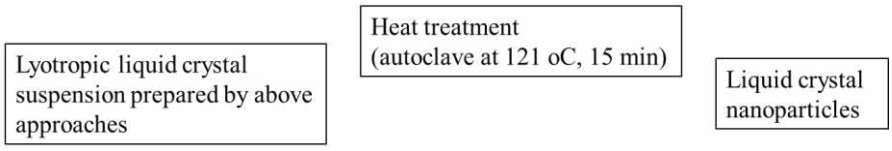
To consider use in mass production, the preparation of the nanoparticles must be cost, time and energy efficient. The production of both hexosomes and cubosomes has a wider variety of preparation methods, each of which will be reviewed briefly in this section.

Top-down preparation: This method for the creation of cubosomes and hexasomes was pioneered in the mid 1990's [64] and remains one of the most popular for production [65]. Using high-energy techniques such as ultra-sonication or shearing, a solution of amphiphiles, drug cargo and a polymeric stabilising agent is agitated sufficiently to form a homogenous dispersion. The stabilising agent used is often pluronic F127, a popular choice to provide steric stability in multiple preparation methods [66-68]. This method can also be carried out using high pressure homogenisation, but will be heavily dependent on the amount of the pluronic polymer and the temperature at which it is prepared[i]. While there is potential for lower energy and more reliable repeatability in shearing, this method is still hindered by the issues liposomes suffer from; large energy cost of production, and the contamination of the final product with unwanted vesicles.



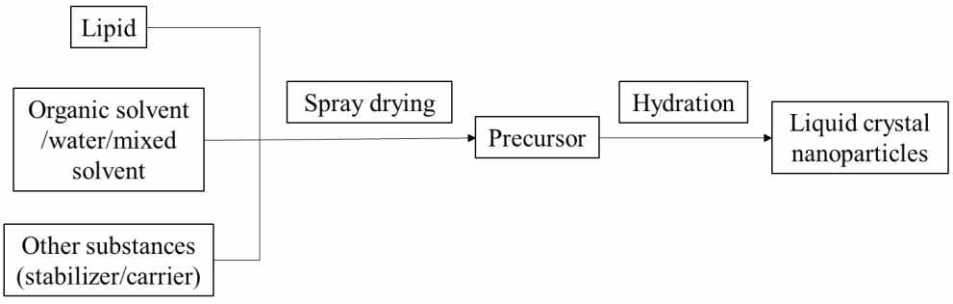
Bottom-up preparation: This method of cubosome production requires the preparation of a solution containing amphiphiles, prospective drug cargo, and, importantly, hydrotropic molecules. These molecules are used to significantly increase the solubility of the amphiphiles in solution, and prevent aggregation once dispersed [73]. The addition of hydrotropes reduces the energy input needed for dispersion and provides a low energy alternative to the top-down approach. In contrast to top-down, the bottom-up method relies on the formation of nanoparticles from solution when agitated, rather than the dispersion of large aggregations. There are multiple benefits to this method beyond the lower energy requirement such as greater stability, as well as smaller cubosomes. The drawbacks of this method are that additional structure such as vesicles are also formed during the procedure, and an intimate knowledge of the phase transitions of the solution is required to produce the desired product. Hydrotropes are also known to cause issues within the human body, meaning their use in the administration of drugs could be rendered nil.

Heat treating: The main advantage of this approach is the ability to minimise the formation of vesicles, an issue that both previous methods could not address. Heat treatment itself is not considered a stand-alone process for the production of nanoparticles, but is one that can be integrated into cubosome production. The procedure reduces the amount of vesicles by promoting the formation of a greater number of cubosomes with greater stability [69]. This provides promising progress in the improvement of sustained release, as vesicles are theorised to interfere with cubosomes [70]. The reason for the success of this method is due to the decrease in surfactant solubility once a specific temperature is surpassed. This allows the vesicles to aggregate, creating more cubosomes while reducing the number of vesicles [71]. A critical drawback to this method is that a temperature of >100 ^{0}C must be reached to activate this effect. Many possible payloads for the cubosomes such as pharmaceuticals, DNA and proteins are temperature sensitive, and will break down when exposed to such extremes, limiting the possibilities of this method.



Spray drying: Spray drying is the process of producing an aqueous cubosome dispersion, and reducing it down to powder form. The process requires a number of specific mixtures for the initial solution. A common example is a lyotropic solution containing glyceryl monooleate (GMO) and hydrophobic starch [72]. A dispersion of these constituents could then be dried to a powder form, known as a 'precursor', which, upon rehydration will reassume the form of the aqueous dispersion. Potential applications for this mechanism could be the most promising of all techniques mentioned. The possibility of oral tablets

that are rehydrated once inside the body would present an ideal method of non-invasive delivery, as well as applications for inhalers. Research has also shown increased efficiency of an anti-inflammatory loaded precursor over the traditional drug, providing potential for increased potency.



Of these methods, there is a huge variety of possibilities and combinations for future application in commercial drug delivery. With a growing research field studying techniques with reduced energy costs, improved potency, greater stability (and therefore circulation times), many of the issues liposomes faced could be tackled in the near future. The mass production of cubosomes and hexosomes may be a long-term goal, however it is also a feasible one. A promising method developed by J. Barauskas et al. using many of the techniques mentioned in this report shows potential for production of customisable cubosomes and hexosomes for loading of cargo by using multiple different lipids to adjust the nanoparticle structure. Rather than using GMO [73, 74], 3,7,11,15-tetramethyl-1,2,3-hexadecane-triol (abbreviated to phytantriol or PtOH)) was chosen initially due to similar phase behaviour in aqueous solution, forming the reverse bicontinuous phase V_2 in excess solution. In a solution of weight ratio PtOH/vitamin E TPGS/water = 1.76/0.24/98.0, cubosomes formed that were both stable and reproducible by applying a heat treating of 125°C for 20 minutes. Boyd et al [54] shows an example of an innovative way to create nanoparticles with lipids not previously used. When comparing this method to those

discussed previously, a combination of top down and heat treatment have been used, demonstrating that these techniques can be combined in different ways to obtain new results. Expanding the method to other lipids: a solution of GMO/F127/water = 1.88/0.12/98.0 produced reverse bicontinuous cubic cubosomes whereas a solution of DGMO/GDO/P80/water = 2.13/2.13/0.74/95.0 produced reverse sponge 'melted' cubosomes and a solution of DGMO/GDO/F127/water = 2.25/2.25/0.5/95.0 produced reverse hexagonal hexosomes.

1.5.4 Methods of characterization

Once the lyotropic liquid crystals are prepared to carry drugs, either as a matrix or as nanoparticles, their characterization can be carried out using a number of techniques used in soft matter physics. The identification of lyotropic phases involves several techniques such as Polarizing optical microscopy, small and wide angle X-ray scattering and electron microscopy. Small-angle X-ray scattering is a well-known technique used to identify liquid crystal phases. [75] It is based on Bragg's Law, $2dsin\theta = n\lambda$, where d is the spacing between different lattice planes, θ is the angle between the incident electromagnetic wave of wavelength $\boldsymbol{\lambda}$ and the layer normal. The intensity of the interference pattern I is a function of angle θ and defining the scattering vector as $q = \frac{4\pi}{\lambda} sin\frac{\theta}{2}$, the intensity I can be expressed as a function of q [1, 75,76]. Polarized optical microscopy is another powerful technique used to identify lyotropic liquid crystal phases given that the resulting birefringent textures each result from a unique director profile. Before the electron microscopy was developed imaging lyotropic liquid crystal structures to nanometer length scales has been notoriously difficult. Techniques such as polarised light microscopy had fallen short due to the sub-micron scale of liquid crystal mesophases, and the interaction of other imaging techniques often causes degradation of

the sample [77]. Traditionally the use of x-ray diffraction, particularly small angle scattering (SAXS) has been the most commonly seen technique for probing at the nano scale, however it can only resolve complex assemblies to a ~10nm resolution [78]. Transmission electron microscopy (TEM) and various other atomic scale imaging techniques have also been applied to liquid crystal imaging but the dynamic and fragile nature of the liquid crystalline sample means that these methods have seen narrow use. This is often because the high energy electrons used in TEM that can significantly impact the mesophases structure due to the effects of radiation bombardment [79]. The lack of imaging techniques has prompted new methods of sample preparation and modification of electron microscopy techniques for observing nano-level structures. There are three main sample preparation methods used to analyse LLCs by TEM; thin film plunge freezing, CEMOVIS and freeze fracture.

Thin Film plunge freezing: This method of preparation is preferred for lyotropic solutions above 80% water concentration [77]. Low viscosity leads to simple preparation making this a favourable technique. A sample of ~3µl of solution is applied to a carbon laced TEM grid, and then suspended between two filter papers before being plunge frozen. The freezing is done at 100% humidity at room temperature, and is plunged into liquid ethane. This method is known to give very clear images without artefacts, however due to the blotting procedure the sample has a tendency to orient parallel to the sample surface and restrict the view of internal domains.

Cryo-TEM of Vitreous Sections (CEMOVIS): This method utilises a bulk approach, using a larger sample than the thin film method. This is to avoid the orientation issues in the thin film method, preserving the structure of the sample. The preparation of the sample

begins with high pressure freezing. The sample is inserted into a thin copper tube a ~300μm in diameter, and then extreme pressure of ~2×10^8 pascals is applied. The tube is then covered in liquid nitrogen to freeze the sample. The frozen sample then undergoes ultramicrotomy (a small slice is taken) using a diamond tipped knife angled at 25° producing a slice ~50-80nm thick for TEM analysis. The application of pressure ensures minimal crystallisation of the water within the sample to preserve the structure as best as possible [77]. For this method cryo-protectant such as dextran is added to solution for percentage concentrations of water of >80% to reduce the water crystallisation further. Due to bulk sampling, issues with orientation are diminished and viable observable areas up to 200nm in diameter are commonly found. Some issues can arise from the ultramicrotomy; compression, grooves and scratches from the knife can affect images.

Freeze Fracture TEM (FFTEM): This method is also classified as a bulk approach. Either high pressure freezing or plunge freezing is first applied, followed by the fracturing of the sample at −160°C. A layer of Pt/C ~4nm in thickness is then applied to the fractured surface, creating a morphology shadow. A second coating of 20nm of carbon is then applied to the morphology shadow to create a film for TEM analysis. After the carbon film has been applied the liquid crystal is dissolved in chloroform leaving the carbon film for TEM analysis. This method is able to achieve high quality images as the resolution is determined by the choice of molecule for the film. Resolutions of 3-4nm are possible in some cases. Despite the high resolution, there is often very little difference between FFTEM and CEMOVIS. Due to the more complicated preparation for FFTEM, CEMOVIS is usually favoured.

1.5.6 Applications of liquid crystal nanoparticles

Drug containing nanoparticles of lyotropic liquid crystals are delivered by a number of routes. Lee et al [53] prepared liquid crystal nanoparticles of ~100nm in size using an energy efficient phase-inversion temperature method. Pharmacokinetic and tissue distribution studies of such nanoparticles having hydrophobic peptide-based drugs showed a five-fold enhancement of bioavailability, sustained release, and liver-specific drug delivery compared to a host-guest complex formulation. Moreover, the fluidity and large surface areas of dispersions of cubosomes and hexasomes make them suitable candidates for topical drug delivery as it provides homogeneous spread and better absorption on skin [80]. Topical applications of carbomer-indomethacin loaded cubosomes, carbomer blank cubosomes and carbomer with an indomethacin water suspension has been reported to show different drug release behaviour and effects on UVB-induced erythema through human test [81]. In an in-vitro permeation study using the drug-loaded hexosomes, the concentration of Cys A in epidermis and dermis ([E + D]) was two times higher than that after application of the control formulation (olive oil solution of Cys A). Similarly, statistically significant enhancement of drug concentration in [E + D] (2.8 times) was derived from in-vivo study. Cubic and hexagonal mesophases and their nanoparticles are also tested for mucosal drug delivery. In this case, precursor systems that form cubic and hexagonal gels by absorption of body fluid in-vivo are employed and sustained release behaviours were observed for over a period of 18 h in-vitro [82, 83]. Swarnakar et al. [84] reported that after application of progesterone loaded hexosomes on the albino rabbit mucosa for 12 h, an enhanced transmucosal flux was observed and that it was fivefold higher than that of progesterone loaded gel and nearly fourfold higher than plain progesterone suspension. Also, lipid extraction phenomena and evident pores were observed in the epithelium of mucosa which indicated a probable intercellular for hexosomes.

Another interesting application of lyotropic liquid crystal nanoparticles is for photodynamic therapy (PDT) [85,86]. In PDT, a photosensitizer that can be activated by light is administered along with drug. The photosensitizer causes selective damage to the tumour and its surrounding vasculature. The limitation of PDT is in administering photosensitizers having low water solubility. Incorporation of photosensitizers in nanoparticles is one of the strategies to overcome this difficulty. Petrilli et al [85] investigated in-vitro and in-vivo penetration photosensitizer Chlorin activated nanoparticles in animal models and confirmed that the nano-dispersion of hexagonal phase enables a higher drug skin uptake compared to the control. They demonstrated a higher bio-distribution of the chlorin derivative in the skin layers of hairless mice compared to the control. The results show the potential of nano-dispersion for the delivery of the photosensitizer into the skin, which is a crucial condition for successful topical PDT.

In addition to improving drug efficacy, liquid crystal nanoparticles are useful to track the intracellular fate of the delivery vehicle and drug cargo. Such simultaneous delivery and tracking needs special multifunctional nanoparticles. Spillmann et al [87] has developed multifunctional nanoparticle based delivery system which provides efficient delivery and allows intracellular fate tracking. The nanoparticles consisted of liquid crystal cross-linking agent, homologue of the organic chromophore perylene, and polymerizable surfactant containing a carboxylate head group. The nanoparticle core incorporated both the fluorescent dye for spectrally tuned particle tracking and encapsulation of amphiphilic and/or hydrophobic agents for intracellular delivery. The carboxylate head groups enable conjugation to biologicals to facilitate the cellular uptake of the particles [88]. Plasma

membrane is an integral part of the cell which regulates endocytosis, trafficking, apoptotic pathways and drug transport. The tracking of such cellular processes in addition to controlled drug delivery via imaging agents is useful. Nanoparticle delivery systems that stays for long time at the delivered site and shows controlled release of cargoes in the plasma membrane while not interfering with regular cellular physiology is ideal for this purpose. Nag et al [89] have developed plasma membrane-targeted liquid crystal nanoparticle formulation that can be loaded with dyes or drugs which can be slowly released from the particle over time. In order to monitor the cellular uptake of liquid crystal nanoparticles, they employed a delivery system for the controlled partitioning of a model dye cargo from within the nanoparticle core into the plasma membrane bilayer. During synthesis of the nanoparticles, the water-insoluble model dye cargo, 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) was incorporated into the hydrophobic nanoparticle core. Conjugation of a PEGylated cholesterol derivative to the nanoparticle surface facilitated the localization of the dye-loaded nanoparticles to lipid raft micro-domains in the plasma membrane in cell. Analysis of DiO cellular internalization kinetics revealed that when delivered as a LCNP-PEG-Chol NP, the half-life of DiO membrane residence time (30 min) was twice that of free DiO (DiO-free) (15 min) delivered from bulk solution. The passive efflux of DiO from the liquid crystal nanoparticle core and its insertion into the plasma membrane bilayer was visualized.

1.5 Summary and Conclusions

In summary, the application of lyotropic liquid crystals as drug delivery systems has been discussed in detail. Liquid crystals help to control the behaviour of drugs in-vivo and maximize the efficiency of medical practice. Lyotropics are the liquid crystals that exhibit phase transitions when in solution and the transitions strongly depend on any change in

condition of that solution. Such flexibility in biochemical fluid of human organs makes lyotropics a promising candidate in drug delivery. Three lyotropic phases are mainly used in drug delivery applications, they are: lamellar, hexagonal, and cubic phases. Each phase has its own structures and properties. The lamellar phase is least viscous because of its layered structure, while hexagonal phase is far more viscous. Cubic phases can be further sub-classified into very different structures. Packing parameter is an important quantity that determines the phase of liquid crystal, and can be calculated from the structure of amphiphile. These bulk phases can further but not necessarily be dispersed into particulate dispersions such as hexosomes and cubosomes with large surface area.

Several promising lyotropic liquid crystal candidates for drug delivery carrier were listed, along with the configuration and general necessary procedures of the experiment. The mechanism of drug delivery can give either sustained release or burst release. The hexagonal phase is a possible candidate for sustained release because of its viscosity. Sustained release is suitable for the condition when need to be gradually absorbed by tissues (such as when the drug can be toxic). Burst release can be achieved by particulate dispersions (such as cubosomes and hexosomes) thanks to their extremely large surface. Such burst release is suitable for the case when drugs need to be urgently absorbed. The influence of parameters such as pH value, temperature and the amount of drugs on the release behaviour has been given. By and large, these parameters primarily have a direct influence on the packing parameter. Then, the phase transition occurs when the packing arrangement is altered. As a result, the release behaviour is consequently changed because the liquid crystal phase has changed.

Biochemical fluids within the body vary from region-to-region. Pathological tissues usually have different pH value than healthy tissues. pH responsive system makes it possible to achieve target selective drug release. By controlling the temperature and loaded drug amount realizes the control of release rate, which account for higher efficiency. Key aspects of the feasibility of widespread usage of these cubosomes and hexasomes nanoparticles are discussed along with the technologies used to identify, characterise and analyse the nanoparticles, and the techniques used to produce them. While there are multiple methods for their production, no standard method can reproduce reliable, repeatable and homogenous dispersions the research in this direction is still in progress. High solubility of these systems makes it possible for patient to take more drugs at once rather than many times. The location of the drug in the mixture ensure that before release the drug is protected against biochemical reaction with surrounding. On the other hand in terms of interaction of drugs and efficacy of delivery more approaches need to be explored. For oral delivery formulations more studies are needed to understand the in-vivo interaction of drug with biological barriers. Although, at this stage, mass production of lyotropic nanoparticle based drug delivery systems is not imminent, future appears promising as medical applications are already being proposed.

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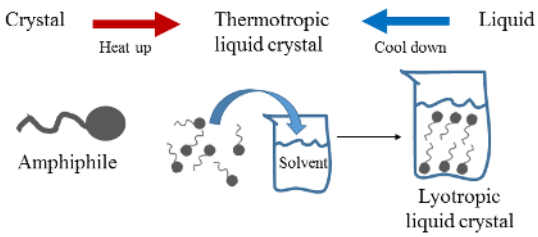

Figure 1: Schematic representation of the formation of a thermotropic liquid crystal on changing temperature and a lyotropic liquid crystal from the self-assembly of an amphiphile in solvent.

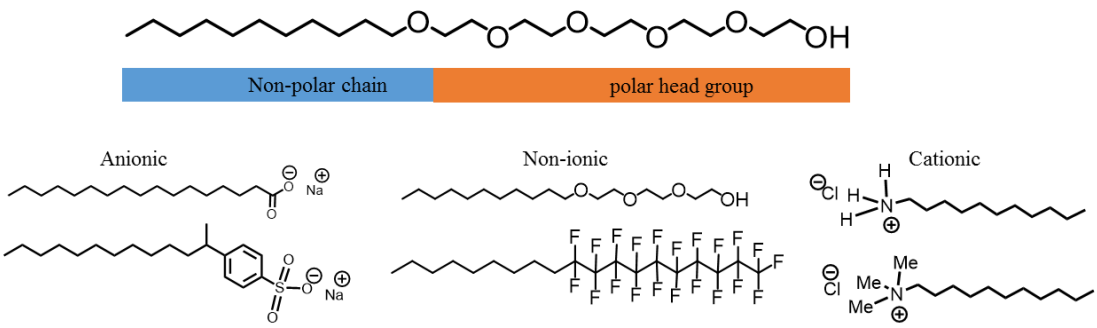

Figure 2: Examples of amphiphilic molecules that form lyotropic liquid crystals when dissolved in solvents.

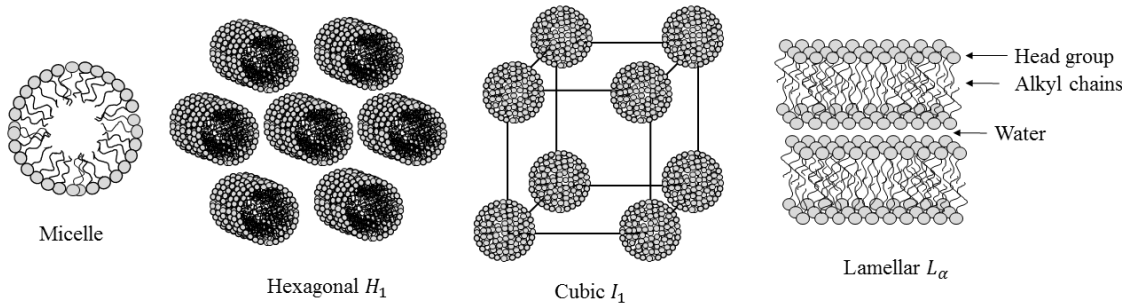

Figure 3: Aggregation of amphiphiles into micelle, and lyotropic liquid crystal phases such as hexagonal, cubic and lamellar phases.

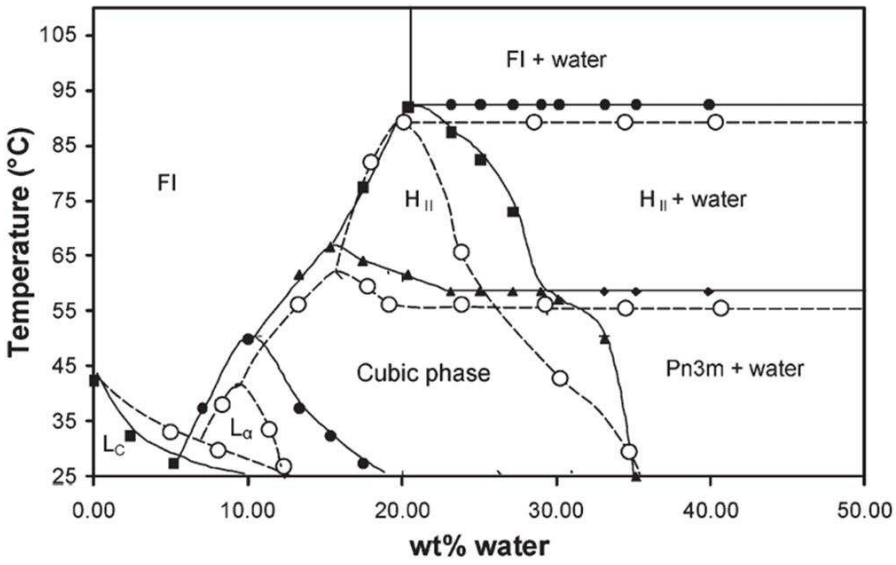

Figure 4: Binary phase diagram of Dimodan U/J and water mixture. Dimodan U/J is an emulsifier made of unsaturated monoglycerides. Dashed curve with open symbols represent Dimodan U/J + water + 5 % glucose solution. Continuous lines with full symbols is related to Dimodan U/J with pure water. Reprinted with permission from Mezzenga et al, Langmuir, 21(14):6165, 2005. Copyright 2005 American Chemical Society.

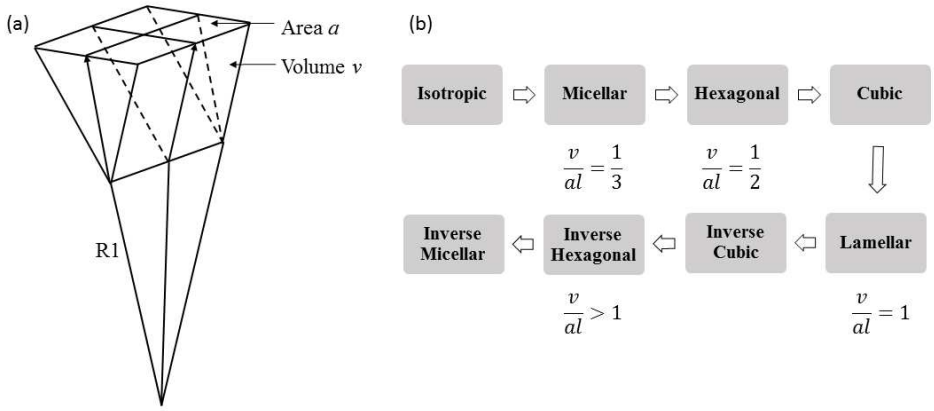

Figure 5: (a) Geometrical representation of an amphiphile. (b) Schematic representation order of formation of the lyotropic liquid crystal phases having different packing parameters.

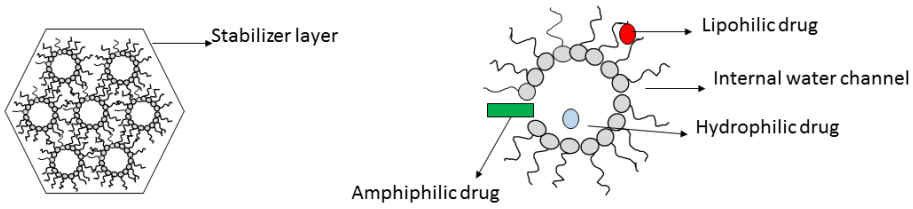

Figure 6: Location of loaded lipophilic, hydrophilic and amphiphilic drugs in a hexagonal phase of inverse topology.

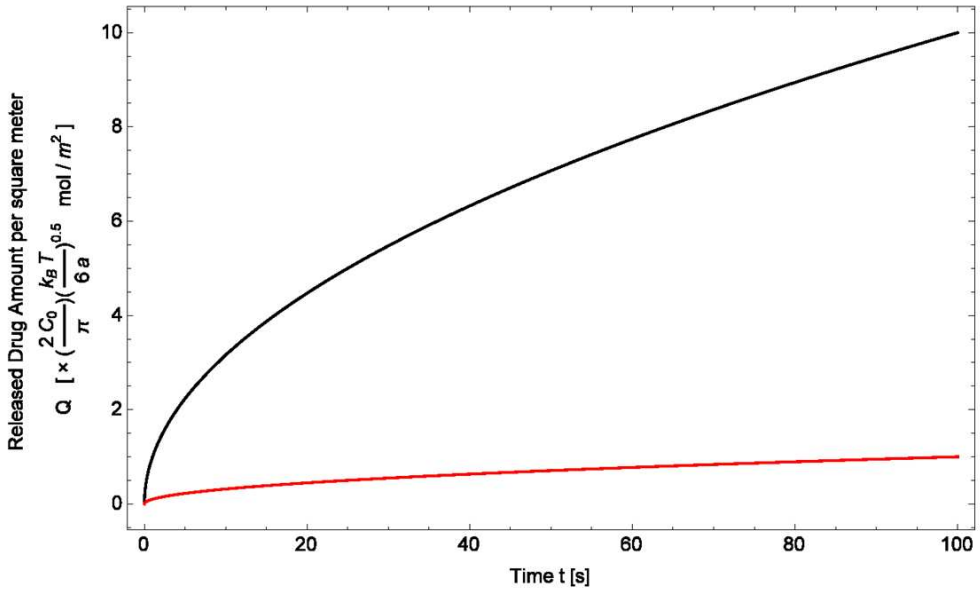

Figure 7: Plot of the amount of drug released per unit surface area as a function of time. The black curve represents drugs released from the lamellar phase following $Q = C\sqrt{t}$. The red curve represents drugs released from hexagonal phase following $Q = \frac{1}{\sqrt{100}} C\sqrt{t}$. $C = \left(\frac{2C_0}{\pi}\right)\sqrt{\left(\frac{k_B T t}{6a}\right)}$.

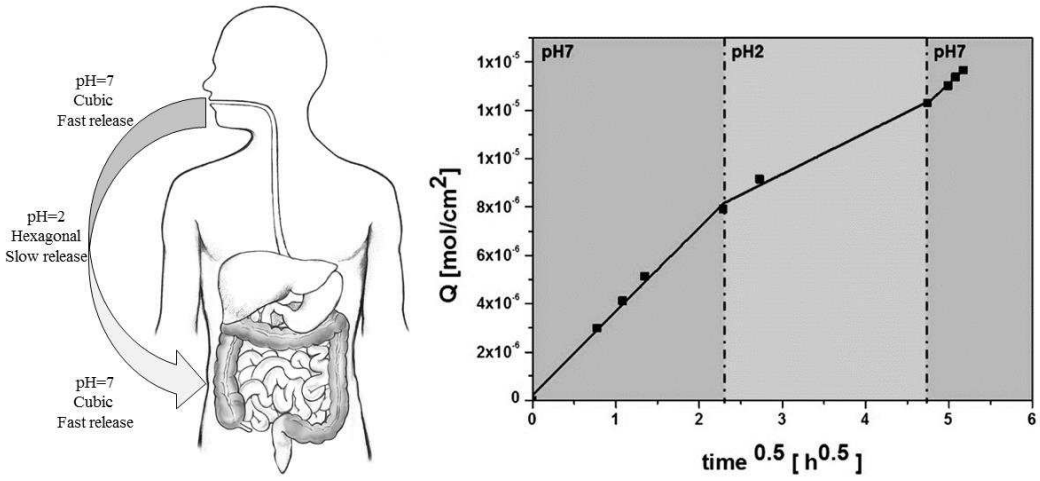

Figure 8: Schematic representation of role of pH in drug delivery. The graph shows the plot of the amount of drug released as a function of time under different pH conditions. Reprinted with permission from Negrini et al., Langmuir, 27(9):5296, 2011. Copyright 2011 American Chemical Society.

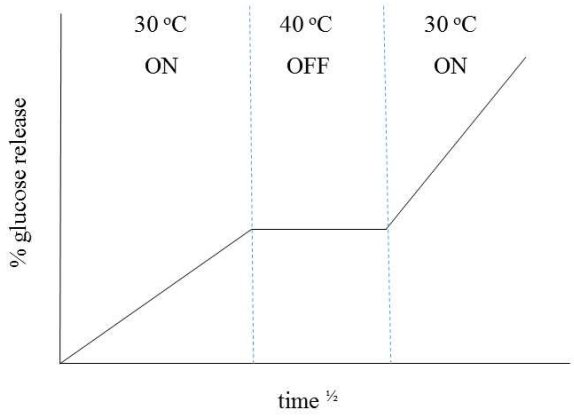

Figure 9: The role of temperature in the drug release process.

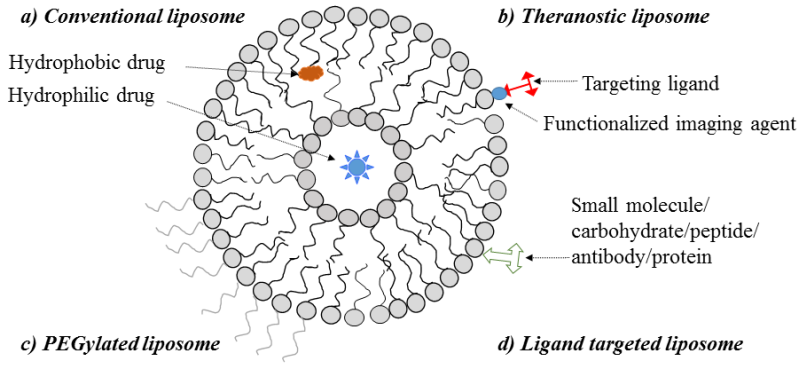

Figure 10: A cross sectional view of the four main types of liposomes. A) Conventional liposome that is able to deliver a variety of hydrophilic, hydrophobic and amphiphilic molecules. B) PEGylated liposome that has been treated with polyethylene glycol to improve steric stability. C) Ligand-targeted liposome that is able to deliver a wide variety of site specific molecules. D) Theranostic liposome which is both for therapeutic and diagnostic purposes. It is able to deliver targeted molecules while also providing an imaging component for helping in diagnosis.

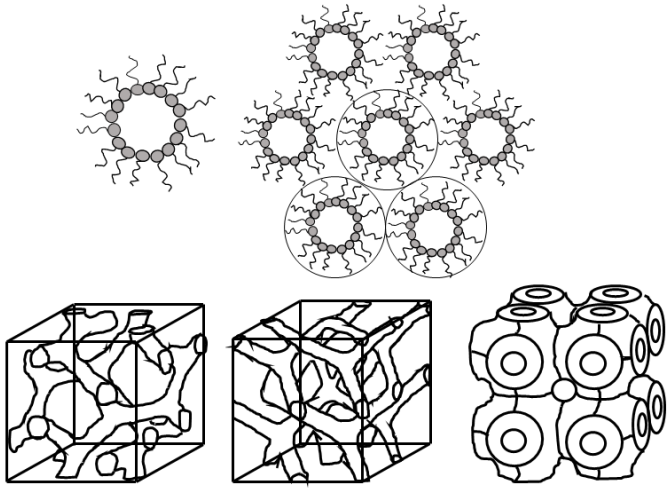

Figure 11: Self-assembled amphiphiles into a reversed micelle, hexagonal columnar structure and the three reverse bicontinuous cubic phases observed experimentally in order of increasing hydration. The columnar structure shows a two dimensional view of the micellar columns. The empty spacing between columns must be minimised to ensure the highest stability of the lattice. The bicontinuous phases from left to right are a Gyroid with spatial grouping Ia3d, Diamond with spatial grouping Pn3m, and Primitive with spatial grouping Im3m.

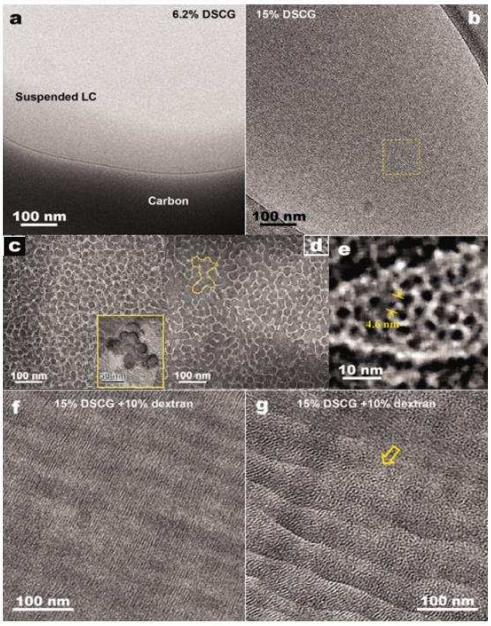

Figure 1: Cryo-TEM images using various methods of sample preparation for varying concentrations of the lyotropic molecule disodium cromoglycate (DSCG). a) Thin film image for 6.2% DSCG, b) Thin film image for 15% DSCG, c and d) thin film images of thermotropic CB7CB, inset c) FFTEM of CB7CB from a different angle. e) Enhanced view of domains perpendicular to the thin film f) CEMOVIS image of 15% DSCG with 10% dextran as cryoprotectant, domains parallel to fracture surface. g) CEMOVIS image of 15% DSCG with 10% dextran, domains perpendicular to fracture surface. Note the image quality of the CEMOVIS images despite the high water %, displaying the effectiveness of dextran as a cryoprotectant. Reprinted with permission from Gao et al, Microscopy research and technique 77 (10) 754, 2010.