Recent advances in drug delivery and medical imaging using colloidal lyotropic liquid crystalline dispersions

Mulet Xavier \textsuperscript{a,b}, Boyd Ben J. \textsuperscript{b}, Drummond Calum J. \textsuperscript{a}

\textsuperscript{a} CSIRO Materials Science and Engineering, Private Bag 10, Clayton VIC 3169, Australia.
\textsuperscript{b} Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville campus), 381 Royal Parade, Parkville VIC 3052, Australia.

*Corresponding author: Calum.Drummond@csiro.au, Fax: +61 3 9545 2059, Tel: +61 3 9545 2050;

Abstract

The overarching goal of this feature article is to review the recent developments in the field of drug delivery specifically involving colloidal lyotropic liquid crystalline dispersions. The development of advanced particles for drug delivery applications is regarded as the next necessary step in the advancement of nanomedicine. An outline of the state of the art in preparation and application of self-assembled nanoparticles to drug delivery and medical imaging is presented in this article. The basic concepts for controlling the nature of particles via the self-assembly of small molecule amphiphiles is covered. Theranostics is an exciting emerging area for this colloidal material class, and the types of therapeutic compounds and medical imaging agents that can be incorporated as well as their methods of preparation is described. The stabilisation and biocompatibility of the colloidal dispersions is also discussed. Finally an overview of lesion-specific active and passive targeting will be presented. Exploiting such a multi-functional drug delivery platform is essential to not only the next generation delivery of bioactive molecules but also in the creation of diagnostic tools.

TOC graphic:
Highlights:
- Development of colloidal dispersions into advanced drug delivery and imaging agents.
- Bioactive and imaging agent effects on particle structure is discussed.
- *In vitro* and *in vivo* applications of the stabilisation sphere/stealth corona.
- Strategies for targeting nanostructured colloidal particles is discussed.

Keywords: cubosomes; hexosome; cubic phase; hexagonal phase; active targeting; passive targeting.

Abbreviations
GMO – glycerol monooleate
MRI – magnetic resonance imaging
SAXS – small angle X-ray scattering
WAXS – wide angle X-ray scattering
DSC – differential scanning calorimetry
Cryo-TEM – cryogenic Transmission Electron Microscopy
CPP – Critical Packing Parameter
EPR – Enhanced Permeation and Retention
ATR-FTIR – Attenuated Total Reflection Fourier Transform Infra-Red
EDTA – Ethylenediaminetetraacetic acid
PEG – Poly(ethylene) Glycol
PEO – Poly(ethylene) Oxide
RGD – Arginine-Glycine-Aspartic acid
PK – pharmacokinetic
TEM – Transmission Electron Microscopy
FESEM – Field Emission Scanning Electron Microscopy
DLS – Dynamic Light Scattering
PDI – Polydispersity index
DNA – Deoxyribonucleic acid
RNA – Ribonucleic acid
QCM – quartz crystal microbalance
DOTAP - 1,2-dioleoyl-3-trimethylammonium-propane
NMR – Nuclear Magnetic Resonance
Nav – neutravidin
5-FU – 5-fluorouracil
Translating the potential of advanced nanomedicine, specifically nanoparticulate nanomedicine, from the bench to clinical applications requires the development of biocompatible, non-toxic matrices, with material properties that can be tuned to the specific application.[1] Whilst polymeric and liposomal drug delivery systems are readily accepted in the drug delivery field, nanoparticles such as quantum dots and carbon nanotubes still retain a high potential for toxicity. It is therefore unremarkable that an approach which potentially exploits naturally occurring molecules for the formation of drug delivery nanoparticles has clear advantages within a “first do no harm” framework for the development of clinical treatments.

The creation of high dimension (2D and 3D) ordered self-assembled nanostructured particles or liquid crystalline dispersions based on naturally occurring lipids, was demonstrated in a seminal article in 1989,[2] but their integration into clinical drug delivery platforms has been over-shadowed by the ubiquitous liposome based on a 1D lamellar liquid crystalline structure. The nanomedicine field is currently dominated by liposomal or polymeric drug delivery vehicles with functionality typically limited to long circulating drug loaded particles.[3]

Particles generated from stabilised amphiphilic materials, are created through the self-assembly of small amphiphilic molecules. Amphiphiles, which contain a hydrophilic headgroup and a hydrophobic hydrocarbon chain region, self-assemble upon addition of water to form structures with long range order, termed lyotropic liquid crystalline phases. Some of these structures, when prepared from amphiphiles with very low aqueous solubility, can be dispersed to form nanoparticles which retain the internal order of the bulk ‘parent’ phase. Typical internal structures include inverse bicontinuous cubic phases (which when dispersed form ‘cubosomes’), inverse discontinuous micellar cubic phase (micellosomes), inverse hexagonal (hexosomes) or lamellar phase (liposomes).[4-7] Cubosomes were first discovered during digestion of fats, the structures observed were later elucidated and found to be bicontinuous in nature.[2, 8] New lyotropic liquid crystalline phases were discovered as recently as 2009,[7] hence the materials and their application to drug delivery are very much in their infancy relative to liposomes. Consequently, whilst all but the most recently discovered phases have been successfully dispersed into nanoparticles, their translation to advanced drug delivery vehicles remains limited.
This feature article outlines and discusses the properties that have driven these versatile materials to be part of the next generation of advanced biocompatible nanoparticles, and focuses on five aspects of the nanoparticle. The first aspect under consideration is the relationship between matrix composition and structure and the effect this has on the therapeutic potential of the nanoparticle. Second, the effects of drug loading on the particle internal structure and the relationship between internal nanostructure and their release profiles will be discussed. The loading capacity of the nanoparticles with respect to medical imaging agents such as MRI contrast, fluorescence or X-ray contrast agents is addressed, as this is essential to the development of multifunctional drug delivery vehicles. The fourth important element is the interface between the nanoparticle and its surrounding environment, which plays a crucial role in both the stabilisation of the nanoparticle and the resulting in vivo interactions. Finally, the potential to functionalise the outer layer of these nanoparticles and how this can be used to impart targeting capability is described.
Transformation and innovation in the field of drug delivery can be achieved by using rationally designed matrices that incorporate a range of properties including sustained drug release targeted to specific sites in the body, simultaneous imaging-based diagnostic tools, and a reduction in adverse side effects from therapeutic compounds. [9-11] As such, one of the fundamental driving forces for nanomedical particle research is the belief that tailored multifunctional nanostructured particles, such as the one illustrated in Fig. 1, have the potential to become a complete framework solution to provide the next generation of advanced drug delivery tools. In order to design advanced smart drug delivery systems, knowledge of the underlying principles that govern the creation, structure, stability and biocompatibility of lyotropic liquid crystalline nanoparticles is necessary.

![Diagram](image)

**Figure 1:** Conceptualised advanced drug delivery and medical imaging nanostructured colloidal particle outlining each of the five elements required for the formation of advanced drug delivery vehicles.

Lyotropic liquid crystalline mesophases are often based around the structure of the lipid bilayer, which forms the basic building-block of biological membranes. A limited list of the most common amphiphilic molecules used for the preparation of mesophases is shown in Fig. 2. The majority of the amphiphiles presented in Fig. 2 can adopt a range of morphologies dependent on the local environment. Variables such as temperature, pressure, pH, salt concentration and salt valency will affect the phase behaviour of these systems.

[5]
Figure 2: Examples of the commonly used lipids for lyotropic liquid crystalline based drug delivery applications.
The abundance of lipids in nature means that lyotropic liquid crystalline phases are ubiquitous in cells. The most commonly observed system in a cellular context is the 1-D flat lamellar structure as displayed by the cell membrane. Whilst many other chemical moieties are present in the fluid mosaic model of a cell membrane, the membrane acts not only as the basic building block of this structure but may also affect the enzymes embedded within.[12, 13] Lamellar phase-forming lipids, such as many phospholipids, can be dispersed in excess water to form liposomes. Liposomes, and specifically “stealth liposomes”, have been successful in drug delivery applications and are currently used in the clinic for several applications.[14] Currently, liposomes are employed for two main reasons, to reduce the proportion of ‘free drug’ to avoid toxicity, and to modify the pharmacokinetic and biodistribution profile of drug. For example, for the delivery of chemotherapeutic doxorubicin, liposomes are used to encapsulate the drug and thereby reduce the cardiotoxic side effect observed with the unencapsulated compound. Myocet® is the non-pegylated liposomal form and Doxil® is the pegylated liposomal encapsulation system for doxorubicin. The pegylated liposomal form provides longer circulation time in the bloodstream, improving delivery to tumor tissues. Several commercial products are available that exploit liposomal technology to improve drug circulation time and reduce side effects as presented in other reviews.[14]

In this article, we will focus primarily on the non-lamellar inverse mesophases, i.e. self-assembled aggregates with interfaces that curve towards the aqueous environment, as these have several advantages over liposomes. Their nanostructure permits loading of a range of agents including hydrophobic, hydrophilic and amphiphilic molecules. All of these compound types can be loaded to high extent due to the presence of significantly higher amounts of lipid per particle compared to liposomes, and very high internal interfacial area, which may be favoured by amphiphilic drugs. The internal structure and its relative rigidity enable different drug or imaging agent types to be loaded into the discrete particles. Mesophase nanostructure has recently been shown to significantly affect compound release rate.[15]
PART I – Nanoparticle core matrix

As mentioned earlier, the liquid crystalline structures formed by some lipids or lipid mixtures are thermodynamically stable in the presence of excess water.[16, 17] The retention of internal phase structure upon dilution and dispersion into particles is necessary for the formation of nanostructured liquid crystalline particles. The phases focussed on herein include the 2-D inverse hexagonal phases and 3-D inverse bicontinuous or discontinuous cubic phases (Fig. 3).[18]

The inverse bicontinuous cubic phases consist of a lipid bilayer that is super-imposed over an infinite periodic minimal surface. Three different inverse bicontinuous phases are observed and these are based on the double diamond, the gyroid and the primitive surfaces. The spacegroup nomenclature used to describe these structures is Pn3m, Ia3d and Im3m respectively. These materials are typically very viscous and difficult to handle; though techniques to overcome this impediment have recently been developed.[19]

The inverse hexagonal phase consists of hexagonally-packed elongated or rod-shaped inverse micelle-like tubes. This phase and its properties has been extensively discussed by Seddon.[5] The inverse discontinuous micellar cubic phase consists of two differently-sized micelles packed in a cubic lattice.[20] An inverse discontinuous micellar hexagonal phase has recently been observed but its novelty means that there is little discussion of it to be found.[7]

Unlike most liposomes which can form colloidally stable dispersions, it is often essential to add a stabilising agent to non-lamellar liquid crystalline dispersions to ensure that the particles do not rapidly flocculate and remain as discrete colloidal particles, typically with a diameter ranging from 100 to 400 nm (Fig. 3).
Figure 3: Structural configuration of self-assembled systems and corresponding cryo-transmission electron microscopy images showing morphology of dispersed nanostructured particles. The CPP values listed are from Israelachvili.[21] Liposomes were prepared using dioleoyl phosphatidylcholine; cubosomes were prepared using phytantriol stabilised with 1 wt % Pluronic® F127; hexosomes were prepared using a lipid mixture comprising phytantriol; vitamin E acetate (9:1) also stabilised with 1 wt % Pluronic® F127; micellesomes comprised oleic acid: sodium oleate (7:3 ratio) stabilised using 0.5 wt % Pluronic® F127 (scale bars 100 nm).
The principal driving force for self-assembly behind the formation of lyotropic liquid crystalline mesophases illustrated in Fig. 3 is the hydrophobic effect, which acts to minimise interactions between the hydrocarbon tails of the amphiphiles and the aqueous environment. This is typically exemplified by cellular membranes that are composed of a wide range of lipids which self-assemble into a bilayer arrangement, the bilayer structure being represented in Figure 3 by the lamellar phase. Variations in the headgroup and/or the tail section of the amphiphile can encourage the formation of thermodynamically stable phases with interfacial curvature of increasing magnitude often reflected by increased dimensionality.

The overall geometric shape of the headgroup and tail of the amphiphile contribute to the phase structure formed in water. The particular mesophase adopted may be rationalised by using the critical packing parameter (CPP), as described in equation 1, which takes into account the chain molecular volume \((v)\) relative to the headgroup area \((a_0)\) and the critical chain length \((l_c)\).[21] It is important to note that these variables are all sensitive to their local environments and will vary in changing conditions such as temperature, pressure, and solution conditions such as pH and ionic strength. For example, increasing the salt concentration alters the Debye screening length and hence reduces repulsions between charged headgroups. This effectively increases the cross-sectional area of the headgroup and hence changes the overall volume distribution of the molecule. Conversely, an increase in temperature leads to an increase in chain splay, increasing the molecular chain volume, and drives the formation of curved phases.[6] For CPP values between 0.5 and 1, lamellar phases are typically observed. For CPP values greater than 1, inverse phases are formed.

\[
CPP = \frac{v}{(a_0 \times l_c)} \quad (Equation \ 1)
\]

Control of molecular geometry to create a particular mesophase under specific conditions has been investigated although only simple structure-relationship based “rules” have been developed.[18] It is known that increased chain unsaturation (e.g. oleic acid, linoleic acid, linolenic acid and arachidonic acid), and introducing chain branching (phytanoic acid) will contribute to the formation of inverse phases through an increase in chain splay.[22, 23] Increasing the hydrophilicity of the headgroup and a reduction in its area will decrease the CPP and thus drive the formation of inverse mesophases. Whilst phase behaviour is complex and often dependent on multiple factors, this type of first order model has proved extremely useful. This complexity has recently been discussed in a review by Fong et al. [18]
Phase diagrams of amphiphiles, particularly those providing information on phase behaviour as a function of temperature and hydration, are essential in the selection of materials suitable for drug delivery applications. The two most commonly used lipids in the preparation of non-lamellar liquid crystalline drug delivery particles are glycerol monooleate and phytantriol. The initially reported phase diagrams of these lipids in the presence of water are shown in Fig. 4. However it should be noted that the phytantriol-water phase diagram in Fig. 4(a) has more recently been shown to be compromised by the presence of “impurities” in the lipid. Fig. 4(b) shows the phase diagram from Dong et al. for phytantriol sourced from a different supplier. For commercially supplied lipids, such as phytantriol, it should be noted that even the presence of small levels of “impurities” may significantly affect the phase diagram and particularly the temperature at which phase transitions occur, such as the inverse bicontinuous cubic to inverse hexagonal phase transition temperature for phytantriol. Addition of a steric stabiliser to facilitate the dispersion of the bicontinuous cubic phase into cubosomes may also induce a phase transition. For example, glycerol monooleate forms a double diamond or Pn3m phase in the presence of excess water as shown in Fig. 4(c). However, when dispersed to form cubosomes using Pluronic® F127 as the stabiliser at 1% w/v of the total dispersion, it adopts a primitive lattice (Im3m).

The choice of lipid from which the basic nanoparticle will be formed will be influenced by the properties that need to be imparted to the nanoparticle. These properties include chemical stability. Glycerol monooleate and the naturally occurring phospholipids can be hydrolysed through acid-hydrolysis or enzyme-catalysed reactions. The enzymes that can degrade these lipids include carboxylesterases and phosphatases. The presence of unsaturation in the lipid chains will also increase their propensity for oxidative degradation. Arachidonic and linolenic chains with several unsaturated bonds are readily susceptible to degradation at room temperature. Other amphiphiles, particularly those without ester linkages or chain unsaturation such as phytantriol are resistant to these degradation pathways. Different lipids will also be able to tolerate different loadings of bioactives, a crucial factor to consider when establishing payloads.

Once the primary lipid used to form the liquid crystalline matrix of the nanoparticles has been established, functionalisation of the nanoparticles can be achieved through the addition of specific amphiphiles possessing headgroups that impact charge, hydrogen bonding or other specific functionality to the interface. The lipophilic tails act to anchor the molecule into the bilayer. Charge can be added to lyotropic liquid crystalline nanoparticles through the incorporation of ionized lipids. Negative charge can be added to nanoparticles by adding an anionic lipid such as...
phosphatidylserine (Figure 2). Positive charge, commonly used to bind DNA or RNA to the surface of liposomes to form lipoplexes, can be imparted to cubosomes through the addition of dioleoyltrimethylammonium propane (DOTAP, Figure 2).[26] Fusogenic lipids such as dioleoylphosphatidylethanolamine can also be added to membranes to try to improve the penetration ability of the particles into cells (Figure 2). Cholesterol can change the rigidity of the membrane and thus affect the stability of the particles; this has been particularly observed for liposomes but its effect on cubosome structure is yet to be reported in detail. Colloidal cubic phase dispersions have been functionalised with Ni(II) chelated in EDTA headgroups of amphiphiles. This approach, used to increase membrane protein bilayer concentration in protein crystallisation trials, has had promising results with increased protein-membrane interaction observed.[27]

Select approaches to functionalisation have been exploited to selectively attach cubosomes to surfaces.[28] For example Fraser et al. functionalised phytantriol cubosomes with a secondary biotinylated lipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000], which enabled attachment of the particles to a neutravidin (NAv)-alkanethiol monolayer at the gold surface of the quartz crystal microbalance (QCM) sensor chip.[29]

Whilst additives have the ability to functionalise the membrane, they may also affect the nanostructure. The loading level of the additive sufficient to induce a phase change will vary between additives and core lipid comprising cubosomes and hexosomes. The extent of the phase change, such as a transition from a cubic phase to an inverse hexagonal phase or an inverse discontinuous cubic, will also be affected by the quantity of the functional additives, their respective packing at lipid interfaces and uptake into the hydrophobic domains. Additives can also be included to tune membrane curvature to select which mesophase will be adopted by the system. For example, to access extremely high curvature phases such as the inverse discontinuous micellar phase, one can use lower curvature lipids (which typically only form inverse bicontinuous phases) and add oil-rich compounds that reduce membrane strain. The reduction in membrane strain permits the membrane to form highly curved interfaces and the magnitude of the curvature can be tuned through variation in the oil loading.[30]

As the complexity of the materials and mixtures can rapidly increase with the number of components, on further addition of drug(s), a stabiliser and different buffers among other possible components, this inevitably leads to multi-dimensional parameter space making accurate prediction of the phase behaviour extremely difficult.
Therefore there is a clear role for high throughput and combinatorial approaches to exploring formulation compositional space.

**Figure 4:** (a) Phytantriol phase diagram adapted from Barauskas et al.[31] (b) Phytantriol phase diagram adapted from Dong et al.[24] (c) Glycerol monooleate phase diagram adapted from Qiu and co-workers[32]
Preparation techniques for nanostructured dispersions

There are two common approaches that can be used for the preparation of dispersed self-assembled liquid crystalline phases: a bottom-up and a top-down approach. Several preparation techniques for the different mesophases have been recently reviewed by Guo et al.\[33\] and these will therefore only be discussed briefly herein.

In a bottom-up approach, the solution conditions are designed to allow self-assembly of lipids to form the particulate mesophase structure from a single phase molecular solution. Typically, the lipid moiety is dissolved in a hydrotrope prior to dilution into an aqueous medium of choice. The dilution causes a rapid decrease in lipid solubility which drives precipitation of the lipid component. If the dilution is established so that precipitation occurs in a cubic phase-water miscibility gap, cubosome dispersions will be formed.\[34\] This system is applicable for a range of amphiphiles and therefore most of the lyotropic liquid crystalline phases can be formed.\[35\] Clearly the presence of a hydrotrope presents significant problems as it will not only affect the phase behaviour of the lipid moiety, it may also make the preparation of complex systems with more than one component more difficult as the individual components will have differential solubilities in the hydrotrope. Furthermore, the presence of hydrotrope in the final preparation may cause further in vivo issues such as toxicity, pyrogenesis and irritation. A similar bottom-up approach has been reported by Abraham et al. in which a micellar solution was first prepared comprising the lipid (phytantriol), stabiliser (Pluronic® F127) and a surfactant (octyl glycoside).\[36\] The concept involved dialysing the micellar solution to selectively remove the surfactant to form cubosomes in situ. However, control of the final particle size distribution, which is critical in applications such as intravenous drug administration or medical imaging applications, using bottom-up dilution-based approaches is also very difficult.

The creation of dispersions from these self-assembled phases is therefore typically performed using a top-down approach. The amphiphile is first hydrated to drive its bulk self-assembly, where water is incorporated into the structure to fully swell the mesophase. In the case of the inverse bicontinuous cubic phases, this first step yields viscous gels that are difficult to handle. The top-down approach requires significant levels of energy to break-up the hydrated bulk phase aggregate and the formation of stable, sub-micron, dispersions of liquid crystalline phases often requires the use of a stabilising moiety. Shear forces can be applied to the bulk mesophase using a variety of
techniques to break up the aggregate, including high pressure homogenisation\cite{37, 38}, ultrasonication\cite{39}, or high speed shearing\cite{40}. These approaches invariably generate heat which may degrade sensitive components that have been added to the multifunctional nanoparticles. Top-down preparation of phytantriol and glycerol monooleate cubosomes typically results in particles that are 200-300 nm in diameter with a polydispersity index of 0.1 to 0.4 when measured using dynamic light scattering.\cite{41} Inverse bicontinuous phases, inverse hexagonal phases, inverse discontinuous phases can all be successfully dispersed with these approaches in the presence of a steric stabiliser to reduce their aggregation and increase their shelf-life in the laboratory. A concise, recent review on the nature of these systems has been written by Yaghmur et al.\cite{42} Examples of dispersions of each of the liquid crystalline phases described above have been provided in Table 1. Recent steps have been made to disperse self-assembled phases in non-aqueous environments such as protic ionic liquids.\cite{43}
Table 1: Compositions and preparation methods for top-down preparation of liquid crystalline dispersions.

<table>
<thead>
<tr>
<th>Dispersed System</th>
<th>Amphiphilic component(s)</th>
<th>Stabiliser used</th>
<th>Phase adopted</th>
<th>Preparation method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>Phosphatidylcholine/Cholesterol – range of compositions reviewed</td>
<td>-</td>
<td>Lamellar phase</td>
<td>Freeze-thaw cycling or high pressure extrusion</td>
<td>[44]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Gd.DOTA.DSA/phosphatidylcholine/Cholesterol/DOPE-rhodamine</td>
<td>DSPE-PEG200</td>
<td>Lamellar phase</td>
<td>Sonication</td>
<td>[45]</td>
</tr>
<tr>
<td>Cubosomes</td>
<td>Glycerol monooleate/Oleic acid</td>
<td>Pluronic® F127</td>
<td>Primitive (Im3m), Double (Pn3m)</td>
<td>High pressure emulsifier</td>
<td>[38]</td>
</tr>
<tr>
<td>Cubosomes</td>
<td>Phosphatidylethanolamine/Glycerol monooleate</td>
<td>Glycerol monooleate -PEG660</td>
<td>Double (Pn3m)</td>
<td>Centrifugation/vortex</td>
<td>[46]</td>
</tr>
<tr>
<td>Cubosomes</td>
<td>Glycerol monooleate/Phytantriol</td>
<td>β-casein β-casein</td>
<td>Primitive (Im3m), Double diamond (Pn3m)</td>
<td>Ultra-sonication</td>
<td>[47]</td>
</tr>
<tr>
<td>Cubosomes</td>
<td>RYLO MG19 glycerol monooleate</td>
<td>Pluronic® F127</td>
<td>Primitive (Im3m)</td>
<td>Vortex, microfluidizer, heat treatment microfluidizer</td>
<td>[48]</td>
</tr>
<tr>
<td>Hexosomes</td>
<td>RYLO MG19 glycerol monooleate/glycerol trioleate</td>
<td>Pluronic® F127</td>
<td>Inverse Hexagonal</td>
<td>Homogenizer</td>
<td>[49]</td>
</tr>
<tr>
<td>Hexosomes</td>
<td>Glycerol monooleate/Tricaprylin</td>
<td>Pluronic® F127</td>
<td>Inverse Hexagonal</td>
<td>Homogenizer</td>
<td>[50]</td>
</tr>
<tr>
<td>Micellosomes</td>
<td>glycerol monolinoleate/tetradecane</td>
<td>Pluronic® F127</td>
<td>Inverse discontinuous micellar cubic (Fd3m)</td>
<td>Ultra-sonication</td>
<td>[30]</td>
</tr>
<tr>
<td>Micellosomes</td>
<td>Glycerol monooleate/Oleic acid</td>
<td>Pluronic® F127</td>
<td>Inverse discontinuous micellar cubic (Fd3m)</td>
<td>High pressure emulsifier</td>
<td>[38]</td>
</tr>
</tbody>
</table>
There are four critical properties of nanostructured colloidal dispersions that need to be evaluated for their application in drug delivery. The first of these is the internal symmetry (or mesophase) of the nanoparticles. The ‘gold standard’ technique for characterizing the internal symmetry in these materials is small angle X-ray scattering (SAXS). The liquid crystalline lattice of the drug delivery particles diffracts X-rays, allowing the determination of the long-range order present inside the particles. The relative position of the Bragg peaks in the scattering profiles of these materials allows the lattice structure of the system to be determined which reveals the type of mesophase present, while the peak positions provides information about the size of the crystal lattice. Typical diffraction patterns for dispersed systems are shown in Fig. 5 below. Dong et al. have provided an extensive review on the subject.[51]
Figure 5: Small angle diffraction patterns and their related integrated plots for dispersions of inverse primitive and double diamond bicontinuous cubic phases, the inverse hexagonal phase and the discontinuous cubic phase. (a) Phytantriol cubosomes (10 % w/v Phytantriol 1 % w/v Pluronic® F127) with $\sqrt{2}, \sqrt{3}, \sqrt{4}, \sqrt{6}, \sqrt{8}, \sqrt{9}$ reflections of the double diamond cubic phase shown. (b) Glycerol monooleate cubosomes (10 % w/v GMO 1 % w/v Pluronic® F127) with $\sqrt{2}, \sqrt{4}, \sqrt{6}$ reflections of the primitive cubic phase shown. (c) Phytantriol hexosomes (10 % w/v Phytantriol, 1 % w/v Vitamin E acetate, 1 % w/v Pluronic® F127) with $\sqrt{1}, \sqrt{3}, \sqrt{4}$, reflections of the hexagonal phase shown. (d) Sodium oleate/oleic acid micellesomes (6.5 % w/v Oleic Acid, 3.5 % w/v Sodium Oleate, 1 % w/v Pluronic® F127) with $\sqrt{8}, \sqrt{11}, \sqrt{12}, \sqrt{16}, \sqrt{19}$ reflections of the inverse discontinuous cubic phase (Fd3m) shown. For this last diffraction pattern please note that the low intensity $\sqrt{3}$ peak is obscured by the broad scattering peak at low of the multi-well plate used as a sample holder.
The low concentration of most dispersions combined with the weak electron density of the components present in liquid crystalline dispersions (typically consisting of C, N, O, H and P atoms with low scattering density) leads to overall low scattering efficiency from such dispersions. Therefore, whilst a laboratory-based SAXS approach is possible, the low X-ray flux available from laboratory sources means that data acquisition is slow and the results are often of limited quality. A synchrotron radiation source (with X-ray flux orders of magnitude greater than laboratory sources) greatly reduces acquisition times and enhances data quality for samples with as little as 1% diffracting material present. The high-intensity of the X-ray beam results in faster acquisition times (seconds compared to hours) with sharp, well resolved peaks and low background. With such techniques it is possible to not only assess the nature of the materials at physiological conditions but also to test whether stimuli-responsive materials respond in the desired fashion. For example it is possible to monitor rapid changes in phase structure as a function of temperature to resolve the mechanism of nanoparticle formation and to compare the effects on internal structure of changes in ionic strength of the aqueous solvent[52, 53]

Thermal analysis of the materials using techniques such as differential scanning calorimetry and thermogravimetric analysis can also provide information as to phase transition temperatures and stability of the materials being tested.[54, 55]

Due to the nanoscale size of the crystalline lattice, transmission electron microscopy (TEM) can enable the visualization of the structure of the liquid crystalline lattice of the structure, as recently reviewed by Kuntsche et al.[56] As these systems only self-assemble in the presence of a solvent (typically water) it is necessary to vitrify the samples to preserve the internal structure, because regular TEM imaging requires the removal of water which would result in loss of the internal structure. Once vitrified the structure of the material can be resolved due to either the characteristic fingerprint (see Fig. 6a) of the phases (such as the inverse hexagonal phase) or the nature of the lattice visualised. Some of the characteristic lattices observed are shown in Figs. 3& 6. It may be possible to image many of the different planes through rotation of the sample to create a tomographic map of the crystalline lattice and thus provide a more accurate assessment of the phase under investigation.[57] One of the main advantages of cryo-TEM is that although a limited population size of the material can be examined, it enables a visual assessment of the sample. Such a visual assessment can also reveal whether vesicles or micelles are present within the sample.
Figure 6: Typical structures observed for inverse hexagonal phase nanoparticles (hexosomes) (a) cryo-TEM image from Fong et al. [18] showing the characteristic fingerprint structure of hexagonally packed inverse elongated micelles, (b) & (c) are field emission scanning electron microscopy (FESEM) showing the 3D nature of the hexagonal phase nanoparticles from Boyd et al. [58] (d) cryo-TEM image from Fong et al. [18] showing cubic phase nanoparticle or cubosome showing internal cubic lattice (e) & (f) FESEM data from cubosomes showing the 3D nature of the nanoparticles by Rizwan et al.[59]

Once the internal structure of the mesophase particles is established, the particle size distribution must be resolved. Techniques for measurement of size and colloidal stability over time are essentially the same as those used for emulsions, and hence are mentioned only briefly below. The hydrodynamic diameter of the particles is typically measured using dynamic light scattering (DLS). Microplate-based DLS instruments have allowed high-throughput approaches to be employed to assess the response of size distribution to a large number of variables including the
quantity of the steric stabiliser required to create dispersions with low polydispersity indices.[25, 60] The surface charge of the particles can also be assessed through zeta-potential measurements.[41]

A light transmission measurement during centrifugation has also been used to assess the colloidal stability of nanostructured dispersions as a function of gravimetric factor, which can be employed to accelerate the migration of the colloidal particles. Changes to the localised particle concentration are reflected in changes in transmission.[50, 61]

The interface of the dispersed particles with bulk solution can be probed by $^{13}\text{C}$ NMR, as relaxation times are susceptible to modifications of the local intermolecular interactions and to molecular dynamics.[62, 63] Several NMR techniques have been employed to investigate the diffusion into or out of inverse bicontinuous phases. Nakano et al. demonstrated the diffusion of Eu(III) ions from the bulk solvent phase into the nanoparticle as the chemical shift of the carbonyl carbon was displaced as the Eu$^{3+}$ species diffused into the mesophase.[64] Momot et al. demonstrated a method to directly measure molecular exchange by examining the different population of $^{23}\text{Na}$ of cubosomes dispersed in saline.[65, 66] A further characterisation technique of interest in examining the binding interaction of bioactives with liquid crystalline phases is attenuated total reflection Fourier transform infra-red (ATR-FTIR) spectroscopy. Although ATR-FTIR is not able to resolve nanostructure, it is able to study headgroup-additive interactions. Using this approach, Mishraki et al. demonstrated the pH-independent manner with which insulin interacts with glycerol monooleate-based hexagonal phases.[67]
PART II Bioactive Loading and Release

In contrast to the success of liposomal drug delivery systems, relatively limited progress has been made on developing other lyotropic liquid crystalline-based advanced drug delivery vehicles. The majority of research in this field still focuses on the characterization of simple particles without investigating function, let alone the multifunctionality that can be exploited with these materials. This is despite the possible advantages of nanostructured nanoparticles over liposomes in terms of drug loading for hydrophobic or amphiphilic compounds as well as peptides and proteins.[52, 68, 69] Patents for a range of applications of liquid crystalline phases and their dispersions have been put in place. Applications of the self-assembled materials range from skin penetration enhancers to controlled release agents.[70-80] Camurus® and Phosphagenics® are two commercial ventures specialising in the development of liquid crystalline phases for bioactive delivery, that have had success in the commercialisation of bioactive delivery products.[81, 82]

The loaded dose of bioactive and the rate at which it is release when exposed to an *in vivo* environment are two of the most important characteristics of drug delivery vehicles.

Bioactive compounds can be loaded into these drug delivery vehicles at different stages of preparation. The localisation of the drug molecule within the self-assembled aggregates is dependent on the inherent properties of the drug such as its lipophilicity, amphiphilicity and size. The drug may associate with the water domain, the lipidic domain or the water-lipid interface of the self-assembled system. The octanol/water partition coefficient can provide an indication of whether the drug will occupy the hydrophobic region of the membrane or reside in the water channels.[83, 84] Engström and co-workers were able to develop methodologies to determine the pH dependant membrane partitioning of several drugs based on their partition behaviour in the presence of cubic phases. They also established that the rate at which partition equilibrium was reached was dependent on the temperature, interfacial surface area as well as agitation.

Although there are always exceptions, it can be expected that as lipidic bioactive compounds are inserted into the lipid membrane, the increased lipidic volume will have a similar effect to the ‘chain splay’ described earlier, increasing the effective hydrophobic volume of the amphiphile and driving the formation of phases with increased curvature towards the water channels.
Complex phenomena can be observed with interfacially-active compounds, where other variables such as compound charge, pH, salt concentration and valency can have significant influence on the mesophase adopted by the lipid in water. An example of this has been presented by Engstrom et al. for the case of the effect of lidocaine and its various salts on glycerol monooleate liquid crystalline systems. Specifically the lidocaine base drives the formation of mesophase with higher negative curvature whilst the hydrochloride salt drives the formation of flat interfaces.[85]

Increasing the loading of water-soluble bioactives into bicontinuous cubic mesophases has been achieved through charge functionalisation of the membrane to enable ion-pair formation. Cationic surfactants have been added to glycerol monooleate cubic phases enabling a ten-fold increase in loading of ketoprofen.[86] The extent of loading was considered to be influenced by the nature of the charged surfactant additive’s headgroup and its concentration within the lipid membrane.

Whilst attempts have been made to predict drug solubility in lipid systems, success has been limited due to lipid microstructure, interfacial effects and competing factors such as properties of the lipid, surfactants and drug.[87]

Liquid crystalline dispersions have been loaded with a number of different drug types (Table 2) and it is apparent that different mesostructured systems have differing thresholds for drug loading above which a phase change is induced. Changes to the lattice parameter of the nanostructure at concentrations below the threshold for a phase transition are often observed. If the drug promotes interfaces with increased curvature, a decrease in lattice parameter is often observed and vice versa. It is therefore important to characterise the phase structure and lattice parameter with increasing drug concentration to gain an understanding of the interaction of the drug with the nanoparticle matrix.

**Table 2:** Examples of composition and administration route of drug loaded lipidic dispersions of lamellar (liposomes), inverse bicontinuous cubic (cubosomes), inverse hexagonal (hexosomes) and inverse discontinuous cubic phases (micellosomes).
<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Micellar/emulsion</th>
<th>Liposome</th>
<th>Liposome</th>
<th>Liposome</th>
<th>Liposome</th>
<th>Cubosomes</th>
<th>Cubosomes</th>
<th>Cubosomes</th>
<th>Cubosomes</th>
<th>Cubosomes</th>
<th>Hexosomes</th>
<th>Hexosomes</th>
<th>Hexosomes</th>
<th>Discontinuous cubic (micellesome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil/Polysorbate 80</td>
<td>estradiol topical</td>
<td>doxorubicin intravenous</td>
<td>doxorubicin intravenous</td>
<td>doxorubicin intravenous</td>
<td>doxorubicin intravenous</td>
<td>cinnarizine Oral</td>
<td>indometacin percutaneous</td>
<td>flurbiprofen ocular</td>
<td>Water soluble vitamin A, dermal</td>
<td>Cyclosporin A, dermal</td>
<td>irinotecan N/A</td>
<td>irinotecan N/A</td>
<td>progesterone mucosal</td>
<td>propofol intravenous</td>
</tr>
<tr>
<td>Tradename (Company)</td>
<td>Estrasorb (Novavax)</td>
<td>Myocet (Zeneus)</td>
<td>Doxil (Ortho Biotech)</td>
<td>[88]</td>
<td>[89]</td>
<td>[90]</td>
<td>[91, 92]</td>
<td>[93, 94]</td>
<td>[95]</td>
<td>[96]</td>
<td>[97]</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Mulet et al. recently reported a high throughput approach to creating and characterizing drug loaded nanostructured dispersions. The loading of a range of drugs in Myverol™ (a commercial version of glycerol monooleate) and phytantriol cubosomes was assessed.[98] Using an automated dispensing and sonication protocol, coupled with a high throughput approach to synchrotron-based SAXS analysis, the effect of incorporation of a wide range of drugs with differing octanol/water partition coefficients, at varying concentrations on the nanostructure was determined. Drugs with high octanol/water partition coefficients, such as progesterone and transretinol, induced transitions to increasingly curved inverse phases such as the inverse hexagonal phase, as a function of increasing concentration. The high throughput approach enabled rapid, efficient establishment of the effects of the drugs on the mesophase of the particles, not possible with traditional techniques in a reasonable time frame. This approach thereby facilitates the rapid discovery of ideal drug loading levels for the desired application, making it possible to incorporate such systems into drug delivery technology pipelines, where rapid production of drug delivery vehicles may be required for screening purposes. Such novel approaches will become increasingly required, as new compounds emerging from drug discovery efforts are increasingly water insoluble and are required to be presented to cells and preclinical models using novel solubilisation methodologies.[99]
Figure 7: Effect of drug incorporation on the lyotropic liquid crystalline phase adopted by phytantriol nanoparticle dispersions at 25 and 37°C. Phytantriol-drug samples were sonicated for 420 s in an automated robotic platform. Data reproduced from Mulet et al. [98]
Drug release

Whilst there is an increasing volume of research towards the loading of compounds into dispersed liquid crystalline phases, little data exist for the release of bioactives from dispersed phases. It is useful to look at the work performed with bulk phases and some of the innovations that have occurred within this field to assess the potential of liquid crystalline colloidal dispersions.

Controlled drug release from bulk phases.

Lyotropic liquid crystalline phase gels have demonstrated potential for use in a range of drug delivery applications including photodynamic therapy.[100] Cubic phase gels, due to their high viscosity, are amenable to a range of administration routes as has been reviewed by Shah et al. [101] Drug release from a range of nanostructured liquid crystalline (inverse cubic and inverse hexagonal) and inverse micellar mesophases has been found to be diffusion controlled but differ between the structures.[15] When the release experiment is performed under appropriately controlled conditions and geometry, the release kinetics can be successfully modelled with the Higuchi model to extract the diffusion coefficient of the drug in the matrix.[102] In this model the rate of diffusion from the a single-sided cylindrical matrix follows a linear dependence with the square root of time (Equation 2).

\[ Q = [D_m C_d (2A - C_d)t]^{1/2} \text{ (Equation 2)} \]

where \( Q \) is the amount of released drug per unit area of matrix, \( D_m \) is the diffusion coefficient of the drug in the matrix, \( A \) is the initial amount of drug in unit volume of matrix, and drug solubility in matrix is \( C_d \) and \( t \) is time.

The diffusion-limited rate of release of acyclovir from a cubic phase system was found to be six-fold faster than that of a commercially available formulation.[103] Release of capsaicin from a glycerol monooleate/propylene glycol cubic phase system was also found to follow Higuchi kinetics and linked to channel tortuosity and phase swelling.[104] Similarly, diffusion controlled release has been observed for salicylic acid from glycerol monooleate cubic phases, and the effect of initial drug matrix hydration was also shown to alter drug release rate. Samples with
low initial water content were found to have lower drug release rate than those entirely hydrated, however it was not perfectly clear whether the result was the influence of transient non-cubic structures being present during hydration.[105] The release kinetics of pseudoephedrine HCl and chlorpheniramine maleate from glycerol monooleate bulk phases were also investigated and shown to be under diffusion control.[106]

Differences between release rates from different liquid crystalline phases has often been mentioned as a potential mechanism to tailor drug release behaviour, but again rigorous studies that investigate the difference between all the mesophases are limited.[107-109] In a recent study, encompassing the majority of the inverse lyotropic liquid crystalline phases, Phan et al. have considered the drug release kinetics of the different lyotropic liquid crystalline phases.[15] It was possible to access the inverse bicontinuous cubic phase, the inverse hexagonal, the inverse discontinuous cubic and the inverse micellar phase through the use of mixtures of Myverol™ and hexadecane to tune the lipid curvature and hence select between the four structures using hexadecane content. The bicontinuous cubic phase was found to exhibit the highest diffusion coefficient of glucose (employed as a model hydrophilic drug) whilst the discontinuous phase displayed a 300-fold decrease in diffusion rate. The release rates from the inverse hexagonal and inverse micellar phases were similar. The principal finding focussed on the fact that the water channel dimensionality or degree of openness to the external environment directly affects the release rates from the relative phases. Whilst such data remains, to the best of our knowledge, untranslated to dispersed phases it is promising that simply changing the mesophase of a material can induce orders of magnitude changes to drug release rate; this point is discussed further in the next section.

Alteration of drug molecular structure to tune release behaviour is one interesting alternative approach that has been explored. The controlled release of hydrophobic drugs from a lipidic cubic phase matrix has been shown through selective alkylation of the model bioactive compound, to alter its partitioning and diffusive behaviour. The release rate was slowed considerably by increasing the length of alkyl group attached to tryptophan, increasing partitioning of the bioactive into the lipid layer, and reducing diffusion in the channels of the aqueous phase.[110]
Stimuli responsive drug delivery systems from bulk phases

Drug diffusion from liquid crystalline phases has been demonstrated to be a diffusion-controlled process primarily affected by the water channel size and the dimensionality of the crystalline lattice.[15, 111, 112] To exploit this, several research groups are developing advanced responsive drug delivery systems that undergo a change in mesophase structure in response to the local environment.

Fong et al. have demonstrated the use of localised temperature changes to control drug release rates on demand, both in vitro and in vivo. As the inverse bicontinuous phase and the inverse hexagonal phase show differential rates of release, changes in the mesophase structure of the materials were exploited to control release rate of the active. The hexagonal phase, with its 2D channel porosity showed slower release of bioactive when compared to the 3D cubic phase. Doping of small amounts of vitamin E acetate (<10% w/w) into phytantriol tunes the hexagonal to cubic phase transition temperature of phytantriol between approximately 65°C to below room temperature. The in vivo proof of concept demonstrated the application of localised heating to a bulk subcutaneously-administered depot (Fig. 8). Control over the phase structure provided control over drug release and absorption kinetics[113] Such research and application may also, in time, be translated to nanoparticles, allowing a controlled ‘switch’ to trigger release of the bioactive compound. The choice of phytantriol as the depot compound was in part due to the fact that whilst GMO and other glycerates or fatty acids such as oleic and linoleic acid are generally recognized as safe foods, they have limited injectable applications due to their high chemical activity which leads on to significant haemolytic activity at elevated concentrations.[114, 115]

Negrini & Mezzenga have exploited pH-sensitive formulations in a similar manner, to control release rates by response of the mesophase structure to pH instead of temperature.[113, 116, 117] Negrini et al. designed systems that are responsive to the difference in pH between the stomach (pH 2) and the remainder of the digestive tract (pH 7). The monolinolein/linoleic acid system formed a cubic phase at pH 7 that released drug at four times the rate of the inverse hexagonal phase formed at pH 2. Although further optimization is required to make the release rates appropriate to in vivo residence times, the system will hopefully have application in protecting against drug induced gastric irritation through targeted release in the colon or intestine.[117]
Light activated mesophases are also receiving increased attention in the literature because the stimulus is highly selective and non-invasive, with potential application in ocular drug delivery. Following from the work described above on developing mesophases that are responsive to directly applied heat, Fong et al. have more recently reported the exploitation of the photothermal effect on NIR irradiation of gold nanorods to control mesophase structure. Using a laser to stimulate the surface plasmon resonance of gold nanorods doped into lipidic phases, the heat generated by the particles was shown to induce a similar reversible switch in mesophase structure as that achieved using direct heating.[116] In an alternative approach, the phase transitions were induced by UV irradiation of mesophases containing a novel photochromic amphiphile, spiropyran laurate, designed to anchor the spiropyran moiety in the bilayer structure of the cubic phase.[118] Photoisomerisation of the spiropyran induced a reversible phase transition to the inverse hexagonal phase, in a similar fashion to the photothermal effect described above. Control of the self-assembly phase of surfactant spiropyrans was first demonstrated by Drummond et al.[119, 120]

Phase transitions can also be induced by other parameters such as dilution (triggered by a change from limited hydration prior to administration, to an excess hydration and sink condition post-administration), salt concentration and valency (particularly Ca$^{2+}$ ions), all of which can affect the headgroup to volume ratio and thus alter the critical packing parameter, driving changes in the mesophase.[53, 121]
Figure 8: (a) Reversible changes in rate of release of $^{14}$C glucose (model drug) from a temperature responsive mesophase (phosphate buffered saline from phytantriol+3% vitamin E acetate) in response to switches in temperature. (b) Normalised plasma concentration profiles for $^{14}$C-glucose after subcutaneous administration to rats. Cubic phase consists of phytantriol only depot and hexagonal/cubic phase consists of phytantriol+3% vitamin E acetate. Dashed line indicates time at which subcutaneous temperature was ‘switched’ from 40 °C to 30 °C. Lines are guides to the eye only. Both figures are adapted from Fong et al. [113]
Dispersed drug delivery systems

The controlled kinetics of drug release from the different bulk phases has yet to be translated to controlled release from the colloidal dispersions. In an article comparing the release rates from the bulk cubic phase to that expected from dispersed cubosomes, the 200,000-fold increase in surface area when going from a bulk phase system to a dispersed system with 200 nm particle size was highlighted as a major factor expected to result in extremely rapid uncontrolled release from the particles.[122] Two important trends in the rates of release and total drug released were observed. The first is that the total amount of drug released was somewhat correlated to the hydrophobicity of the compounds used. It can be expected that complete release of water-soluble drugs would be into the aqueous media would be expected upon dispersion and dilution, whilst the more hydrophobic bioactives will be retained in the drug delivery vehicle due to partitioning effects. Second, the rate at which this initial compound was released was independent of the hydrophobicity of the bioactive, at least on the timescale of the measurements. The increased surface area of the dispersions when compared to that of the bulk phases led to rapid or burst release.[123] This leads to the inevitable conclusion that controlled release has yet to be unambiguously achieved from dispersed liquid crystalline phases containing solubilised bioactives intended for intravenous injection, except in the case of liposomes, where permeability rather than diffusion controls drug retention in the particle.

Examples of nanoparticle types, their compositions and their administration routes are highlighted in Table 2. There are as yet no dispersed lyotropic liquid crystalline phases in the clinic other than liposomes. This is not surprising as there are only very few examples of the application of nanostructured nanoparticles in vivo in the academic literature. One of these examples is the intravenous injection of cubosomes/propofol dispersion.[97] Clinical formulations of propofol require the presence of high levels of triglycerides as a solvent for the drug, which can become problematic over extended infusion periods. Cubosomes are thought to have high drug loading capacity relative to the levels of lipid present, and have been shown to have positive effects on the pharmacokinetics properties and the measured pharmacodynamic parameter.

Colloidal particles of soy phosphatidylcholine and glycerol dioleate were also shown to prolong the circulation of large molecules. Loading of a peptide hormone active, somatostatin, into these polysorbate stabilised
lyotropic liquid crystalline particles showed circulation half-life in vivo. A ten-fold increase in the half-life of intravenously administered peptide was observed from “unprotected” peptide compared to that of somatostatin encapsulated into the liquid crystalline colloidal particles.[124]

Sustained absorption of drug after oral administration of cinnarizine in cubic liquid crystalline particles has been demonstrated.[88] The digestibility of the lipids comprising the particles played an important role in particle internal phase stability, their retention in the gastric compartment and consequent slowed rate of drug absorption. Glycerol monooleate, a digestible lipid provided no significant sustained release effect beyond eight hours after administration. In contrast, phytantriol, which is not digestible, showed a sustained release effect over greater than 48 hours. A similar result has been reported using selachyl alcohol, a non-digestible which forms inverse hexagonal phase in excess aqueous environments.[125] Administration of cinnarizine loaded into the hexosomes provided similar pharmacokinetic profiles to administration in phytantriol cubosomes. These results echoed earlier studies with the bulk phases of these lipids, and earlier work using the bulk inverse hexagonal phase of oleyl glycerate.[126, 127] It is important to recognise in the context of understanding the potential of controlled release using cubosomes and hexosomes that the sustained absorption of drug was not attributed to slow release from the particles, but to the fact that the particles were retained in the stomach, which for a poorly water soluble drug such as cinnarizine does not constitute a sink condition – therefore drug was retained in the particles until the surrounding ‘free’ concentration was reduced through gastric emptying and/or absorption.

Lian and co-workers recently demonstrated that bioavailability of silymarin was enhanced at least 3-fold following oral administration of a formulation consisting of GMO-Pluronic® F127 based colloidal particles.[128] Other recent work by this research group has shown almost two-fold improved bioavailability for cyclosporine following oral administration in beagles.[129]

Spray drying preparative methods for the formation of protein delivery cubic phase particles were shown to be possible.[130] Whilst some sustained release from a glycerol monooleate matrix was obtained, the preparation and hydration of the system tested did not permit the formation of stable colloidal dispersions. It is thus difficult to compare these findings with other release data from lyotropic liquid crystalline colloidal systems. The preparations,
intended for mucosal delivery, showed some promise, when placed as a solid or wax on a membrane, as sustained controllable release in the presence of several adjuvants. [130]
Self-assembling prodrugs as drug delivery vehicles.

Recognising the difficulties associated with both drug loading and drug release, an alternative approach would be to engineer systems where the drug is part of the lipid from which the particles self-assemble. One further limitation, particularly for anti-cancer drugs, is the high level of metabolism and efflux at the target cellular level that often limits drug effectiveness. Amphiphilic prodrugs could conceptually be designed to not only control release rates but also influence the rate at which the drug is metabolised. This approach has the potential to avoid the burst release from colloidal drug delivery systems. A molecular engineering route has been recently reported, to create tumour-activated, amphiphilic prodrugs that can self-assemble.[131] Gemcitabine-squalene derivative was developed in Couvreur’s research laboratories.[132-135] In a series of research articles they demonstrated the self-assembly properties and efficacy of this novel compound approach.

Sagnella et al. and Gong et al. have investigated the effects of creating prodrugs that can self-assemble with varying nanostructures and assessed the performance of their novel compounds against tumour xenografts.[55, 136-138] It was demonstrated that the oleoyl, phytanoyl and palmitoyl lipid derivatives of the commercial 5-fluorouracil (5-FU) prodrug, capecitabine, self-assembled to form nanostructured particles. The unsaturated and branched oleoyl and phytanoyl chains yielded cubic phase dispersions at physiological conditions, and the palmitoyl derivative gave rise to solid lipid nanoparticles (crystalline lamellar phase). The colloidal particles were shown to be highly effective, target-activated, chemotherapeutic agents, with significantly enhanced efficacy over capecitabine. The enzymatic release rate of drug from the colloidal particles was found to be dependent on the nature of the lipid chain and the internal nanostructure of the particles, leading to a controlled sustained bioactive release rate (Fig. 9). The lipid-based prodrug colloidal particles were shown to slow the growth of a highly aggressive mouse 4T1 breast tumour, and halt the growth of a human MDA-MB-231 breast tumour in mouse xenografts.[138] Systemic toxicity was reduced through a three-step enzymatic activation of the prodrug to 5-FU, with the final step occurring preferentially at the tumour site.
**Figure 9:** A Effect of aliphatic chain length on its removal rate from prodrug by carboxylesterase. Indexing refers to structures shown in B where (i) is capecitabine, (ii) oleoyl-capecitabine derivative, (iii) phytanoyl-capecitabine and (iv) palmitoyl-capecitabine. Panel C shows the cryo-TEM data from the nanoparticles with large particles solid lipid nanoparticles seen in (i) for the palmitoyl derivative, and cubic structures seen in (ii) oleoyl derivative and (iii) phytanoyl derivative. Figure is adapted from from Sagnella *et al.* [138]

An analogous approach is being developed where lipids are being functionalised with nucleosides, nucleotides and oligonucleotides as DNA or RNA binding domains. Whilst in its infancy such an approach may provide an alternative to charged condensed systems for siRNA or gene therapy delivery using self-assembled nanoparticles. [139-141]
PART III – Liquid Crystalline Colloidal Particles as Carriers for Imaging agents

True multifunctionality in nanomedicine requires a diagnostic or tracking element of the particles, to provide spatial and temporal information on the localisation of the colloidal dispersions. Providing a diagnostic capability, or the ability to visualise the fate of the nanoparticles in vitro and in vivo, is a critical part of the development of advanced functional colloidal particles. Such an approach will contribute to the understanding of nanoparticulate behaviour in a biological context. This is of particular relevance as the ability to account for the fate of the full administered dose of bioactives is crucial to proving the efficacy and safety of the colloidal particles.[142, 143] It is also important to assess in vitro, the localisation of the nanoparticle in a cell when it encounters specific tissue types, as this permits the tuning of the cell penetration of the nanoparticle, its cellular fate and the release of bioactive. There are still few colloidal particle-based imaging systems approved for use in the clinic due to the non-trivial necessary regulatory hurdles that are in place.[144]

One of the research areas with a large potential for growth within the field of nanomedicine is that of medical imaging. Non-invasive imaging techniques such as Magnetic Resonance Imaging (MRI) are commonly exploited in the clinic. To improve the sensitivity of these instruments, contrast agents are often used to improve the resolution and diagnostic ability. These contrast agents must, apart from being able to generate a good signal contrast, have low toxicity and be easy to administer intravenously. The effectiveness of MRI contrast agents is assessed through relaxivity measurements.

It is common for colloid based systems to be used as liver contrast agents due to their rapid uptake by the reticuloendothelial system (See Section 4 for further discussion). The examples presented here focus on the creation of colloidal systems as contrast agents in MRI. Other medical imaging techniques can also benefit from contrast agents or specific ligands such as X-ray imaging and Positron Emission Tomography.

Currently used contrast agents already in the clinic typically use chelated gadolinium, however gadolinium is highly toxic as a free trivalent ion. [145] [146] To address these toxicity issues and to improve the performance of contrast agents, several attempts have been made to incorporate gadolinium-chelating lipids into cubosome systems. Liu et al. and Conn et al. formed soaps through the chelation of Gd$^{3+}$ to oleate and phytanate respectively and incorporated these into colloidal particles to assess their performance. [147-149] Lanthanide salts of oleic acid and in particular those of gadolinium showed significant effects on proton relaxivities in water, whilst the europium (III) salts exhibited strong luminescence.[147] When loaded into Myverol™-based cubic phases, the gadolinium oleate
salt showed a marked increase in relaxivity, with the highest relaxivities occurring at 1 wt % loading.[149] The relaxivities measured were 4 to 7 times higher than those of a commercially available contrast agent.[149] Particulate dispersions of gadolinium(III) phytanate were found to display proton relaxivity values comparable to those exhibited by a clinically used contrast agent for magnetic resonance imaging and a europium(III) phytanate colloidal dispersion showed potential as a luminescent imaging agent.[148]

Moghaddam et al. used a multidentate approach to chelate Gd$^{3+}$ to a EDTA-functionalised oleoyl and phytanoyl lipids. Increased metal ion chelation should result in a reduced level of cytotoxic free metal ion. They found that the addition of the hydrophobic domain enhanced the relaxivity values from Gd and Mn significantly when compared to the systems without hydrophobic groups.[150, 151] Analogous approaches using diethylene triamine pentaacetic acid headgroups have also been used.[152]

Using metal-free nitroxide lipid contrast agent systems Muir et al. created cubosomes that could be loaded up to 12 wt % with a nitroxide lipid without compromising the cubosome internal structure. They successfully used these nanostructured colloidal particles as contrast agents in animal models. At a specific concentration threshold, a phase transition to the inverse hexagonal phase was effectuated for both phytantriol and Myverol™ based systems. Relaxivities were found to be comparable to those achieved using gadolinium complexes such as Omniscan™, despite the absence of heavy metal coordination complexes. In this article a relationship between mesophase of the colloidal particles and their performance as an imaging contrast agents was established. This was observed as the incorporation of nitroxide lipids into the lipid layer led to a greater enhancement of relaxivity in the inverse bicontinuous cubic phase compared to the measurements obtained in the inverse hexagonal phase formed at high nitroxide lipid loadings.[153] Additionally, phytantriol particles were found to have increased cytotoxicity compared to the Myverol™ based colloidal particles.

In the development and assessment of imaging agents loaded into colloidal particles, it should be noted, as confirmed in the case study above, that confining the imaging moieties to the differently nanostructured particles may have significant effects on their respective performance. For example water confinement in a certain phase may affect MRI agent performance, and quenching may occur more rapidly for fluorophores when confined inside a particle relative to the solvated fluorophore. The change in performance of all the imaging agents as they are inserted into a lipidic environment should be assessed, as optimal loading will invariably depend on several variables for
example level of polar group hydration, self-quenching, local pH and membrane pressure. These performance assessments will allow the determination of the optimal loadings for both *in vitro* and *in vivo* experiments.

The lipidic nature of the nanoparticles presented herein means that *in vitro* imaging and tracking of the systems can be performed through the use of fluorescently-labelled lipids or cholesterol derivatives.[154] Hybrid inorganic-organic systems can also be used by incorporating quantum dots within the nanoparticles. This may be best achieved by using oleoyl or similarly functionalised q-dots to confine the particles to the hydrophobic portion of the membrane.
PART IV Steric Stabilisation Layer or Stealth Corona.

Steric Stabilisation

Generally at the interface of a colloidal particle and its surrounding environment is a stabilisation layer. The ultimate utility of nanostructured particles for drug delivery and medical imaging will depend on effective colloidal stabilisation with retention of internal nanostructure during storage and following in vivo administration. Steric stabilisation has been associated with polymer encapsulation and protection of colloidal particles for several decades.[155]

As the lipids used in these dispersions are typically uncharged and their headgroup structure does not impart steric stabilisation for particles once in solution, particles will rapidly flocculate shortly after dispersion. To form nanostructured particles with long term stability, the lipids are typically dispersed in the presence of a steric stabiliser. The “kinetic” stability afforded by the presence of these stabilisers can give a shelf-life of several years to the dispersions.

Although several alternative types of stabilisers have been assessed, non-ionic block copolymers are generally used to impart steric stabilisation because at low concentration and they have been found to yield lipid particles with sub-micron size with long shelf-life and limited effect on the mesophase of the lipid nanoparticles.[25, 60]

The two main elements of an effective steric stabilisation layer are the length, or more accurately volume & density, of the stabilisation layer and the adhesion strength of the stabilizer onto the nanostructured particles. The structure-property relationships governing stabilisation of these unique nanomaterials have yet to be properly established. The molecule of choice to form a steric stabilisation layer in aqueous media is poly(ethylene glycol) or poly(ethylene oxide) (PEO). This polyether has a monomeric repeat unit mass of 44 g mol\(^{-1}\), a 100 repeat polymer thus has a mass of 4.4 kDa.[156] Both the chain length and chain density of PEO determine the thickness of the stabilizing layer.[157, 158] At varying surface concentrations, the polymer can adopt mushroom or brush configurations which will greatly affect its effectiveness as a stabilizer.[156] The effectiveness of the PEG layer, logically, increases with increased PEG density and thus increased PEG overlap.[158] Wattendorf and co-workers carefully outline the physicochemical properties that render PEG the polymer of choice to make surfaces that are protein repellent and resist cellular recognition[156], and these tend to correlate with steric stabilization capability.
The stabilising effect of PEO can be attributed to two parameters.[155] The first parameter is a mixing effect driven by the balance of the PEO–solvent interaction relative to that of the inter PEO-PEO attractive forces. PEO has very high affinity to water which contributes to its solubility. The second parameter is the magnitude of the restriction of the chain motion which contributes to the entropic effect. This effect provides a barrier to flocculation when two stabilised particles are in close proximity. This polymer has several other advantages that make it the benchmark for steric stabilisation including high water molecule binding, flexible polymer chain and low toxicity and immunogenicity. For lyotropic liquid crystalline phases the PEO layer is provided through non-covalent attachment of a triblock copolymer consisting of poly(ethylene oxide)- poly(propylene oxide)- poly(ethylene oxide) (commercially known as Pluronic®) as shown in Fig. 10.

**Figure 10:** Schematic of a Pluronic® steric stabilisation layer around a cubic phase colloidal particle. The poly(ethylene oxide) layer is shown on the outside of a dispersed lipid particle and is attached through a hydrophobic domain (poly(propylene oxide)).
Stealth Corona

Immunogenicity or the development of an immunological response to foreign particles will inevitably occur when colloidal particles are introduced to the circulatory system or tissue. In vivo, these foreign particles can be cleared by the reticuloendothelial system (RES) which will rapidly remove any unprotected colloidal particles from the circulatory system.[159] One of the first steps required to reduce this clearance is to reduce particle opsonisation or non-specific adherence of serum proteins to the surface of the foreign nanoparticle.[160] A reduction in opsonisation leads to increased circulation time and in turn improves delivery of the colloidal particle to the site of interest. This increased circulation time should also be effective if an active targeting moiety is also present to give extended time for antigen recognition. Based on this seminal 1987 work a large range of other stealth particles have developed.[161, 162]

Klibanov and co-workers demonstrated over 20 years ago that “amphipathic polyethylene glycols effectively prolong the circulation time of liposomes”. This gave rise to the first extended circulation or “stealth” particles.[163] The PEO layer was discovered to be the most effective way to prevent the immune system from clearing the particles. This is thought to occur through a reduction in the adsorption of proteins on the surface of the colloidal particles, enabling the particles to evade phagocytosis.[163, 164] The immune system is thus prevented from removing the particles before they can be effective. For therapeutic particles the development of this “stealth corona” is important, as passive targeting (see Section 5) can be increased as the colloids have extended time for accumulation and thus a greater therapeutic opportunity.[165, 166] [14, 163] The effectiveness of the stealth corona in masking the colloidal particles will be dependent of both the thickness of the layer and the density of the polymer chains at the particle/aqueous environment interface (as illustrated in Fig. 11).

The steric stabilisation layer is therefore not only key to successfully dispersing the bulk lyotropic liquid crystalline phases it also creates a stealth layer around the nanoparticles reducing undesirable biological interactions.
Figure 11: Schematic of stealth corona variation around a cubic phase colloidal particle. The thickness of the protecting layer and its density will both affect the effectiveness of the corona to impart protection from the immune to the colloidal particles.

Aggarwal et al. have concisely reviewed the effects of plasma proteins on nanoparticle biodistribution.[167] They outline several nanoparticle properties that influence protein binding to the particles. Specifically: surface charge, hydrophobicity and particle size/morphology. Smaller particles have a larger surface area and therefore have greater propensity for protein attachment in vivo. Neutral particles have been shown to have slower opsonisation rates than charged particles as outlined in a review by Owens and co-workers.[168] In this article, the role of particle hydrophobicity is also discussed; hydrophobic particles have higher opsonisation rates than hydrophilic ones.[168] PEO coatings have been shown to reduce protein binding compared to unmodified surface and this has been found to apply to Pluronic® F127 modified surfaces also. Particles with shorter circulation time typically aggregate in the liver and spleen whilst pegylated particles are retained for longer periods within the circulatory system.

Whilst extensive studies of stabilisers for liposomes have been performed, limited investigations assess the current steric stabilisers of dispersed lyotropic liquid crystalline particles. A few compounds or particles have been tested as alternative stabilizers for lyotropic liquid crystals nanoparticles. Examples of other stabilizer classes examined include clay particles, silica particles, β-casein, polysorbates, modified cellulose and ethoxylated phytosterol.[47, 60, 61, 169-172] A commercially available class of stabilising agent that performs the dual role of
steric stabiliser and “stealth” agent are the poly(ethylene glycol) phosphatidyl lipids. They anchor into the lipid bilayer through a hydrophobic diacyl moiety and possess a multi-PEG unit headgroup that provides the stabilisation and stealth element.

Historically, the steric stabiliser most used for the preparation of colloidal lyotropic liquid crystalline particles has been Pluronic® F127. F127 is a triblock copolymer consisting of poly(ethylene oxide) – poly(propylene oxide) (PPO) – poly(ethylene oxide) with PEO chains of 100 units and a 65 unit PPO domain. The PPO domain is thought to attach or anchor to the cubosomes whilst the PEO domain is hydrated and contributes to the steric stabilisation nanoparticles. A typical concentration required to ensure good long term stability of the nanoparticles is 1 wt. %.[25]

With the advent of high throughput techniques in the preparation of cubosomes, it has recently been possible to increase the compositional space that can be investigated to assess the performance of a range of steric stabilisers. In a recent study performed by Chong et al. assessing the Pluronic® class of steric stabilisers, it was demonstrated that the hydrophobic part of the steric stabiliser, the PPO block, is crucial to the anchoring level of the stabiliser to the cubosomes and imparting long-term stability.[25] The extent of adsorption of the polymer on the surface of the colloidal particle is directly related to the length of the hydrophobic poly(propylene oxide) unit. A longer chain will lead to increased adsorption and stabilisation potential. The minimum hydrophobic domain length of the block copolymer found to successfully stabilise particles was 40 PPO units.

It is clear that each steric stabiliser, especially their lipid-anchoring domain, will affect the different lipids that make up the particle’s ordered structure to varying levels. This is particularly evident when comparing the stabilisation of phytantriol and that of glycerol monooleate. When dispersed in the presence of Pluronic® F127, phytantriol retains its liquid crystalline behaviour as observed in the bulk phase. It remains in the double diamond inverse bicontinuous cubic phase in excess water at 25°C, with a lattice parameter similar to that of bulk phase material. Dispersions based on glycerol monooleate as the main constituent in the presence of F127 undergo a phase change when compared to bulk mesophase in similar conditions.[38] The phase adopted by bulk glycerol monooleate at 25°C in excess water is the double diamond phase but dispersed in the presence of Pluronic® F127 it adopts a primitive lattice arrangement. This points to differential effects of stabilisers on the observed mesophase which must be taken into account during the design, preparation and assessment of colloidal particle dispersions.
The minimum effective hydrophilic PEO domain length to create colloidal dispersions was found to be 19 units, with at least 100 units required for excellent stabilisation and enhanced particle shelf life as summarised in Fig. 12. [25]

Figure 12: Effect of Pluronic® on the ordered structure of colloidal particle dispersions. Lyotropic liquid crystalline phases for dispersions of (a) phytantriol, and (b) monoolein when stabilised using the Pluronic series. Adapted from Chong et al.[25]
Recent, as yet unpublished, studies performed by us confirm that there is little existence of cubosomes in plasma. We observed destruction of the cubosomes internal structure in the presence of whole plasma using synchrotron SAXS. Similar observations were made by Leesajakul et al. using gel filtration. As well as lipolytic activity of whole plasma towards glycerol monooleate based cubosomes, particle integrity was also affected and cubosomes were seen to be destroyed.[173] They observed that the particle size decreased in the presence of high density lipoprotein. Low density lipoprotein were seen to fuse with cubosomes and albumin stripped the glycerol monooleate from the cubic phase particles. They did hypothesise that some cubosome remnant particles were responsible for the long circulation time of incorporated fluorophores.

The research published by Leesajakul et al. leads us to surmise that the current knowledge on the in vivo fate of colloidal lyotropic liquid crystalline particles is relatively limited, and that little is known about the effectiveness of Pluronic® F127 to provide a stealth corona for cubosomes. Further research in this domain is warranted to establish the nature of the stealth corona required to protect the particles from being cleared from the blood stream. A lyotropic liquid crystalline dispersion with prolonged circulation time in blood can be assessed with several quantifiable pharmacokinetic (PK) parameters. These include: an increased half-life, reduced clearance and an increased mean resident time and vice versa for a formulation that exhibits poor circulation times. Tissue accumulation is also crucial in determining the efficacy and side effects of a drug delivery vehicle. Several liposome -products that use this approach to extended circulation time have been approved including the chemotherapeutic doxorubicin liposomes (Doxil). It must be noted that it is important to establish the PK profile of nanomedicines as typically, the PK profiles of the parent and encapsulated compounds differ considerably.[174] Both the PK and biodistribution of the colloidal particles will have significant effects on both efficacy and just as importantly side-effects of bioactive substances.

Since the inception of particles with stealth coronas, extensive studies have been performed on the ability of PEG molecules to shield the drug delivery vehicle from the immune system. Applying these developments to nanostructured colloidal dispersions should contribute to their increased efficacy.[175] It has been demonstrated, in mice, that stealth coronas with longer PEG lengths reduce the clearance rate of liposomes form the blood stream. A similar effect is observed with increasing PEG density on the particle surface increasing circulation time in vivo.[176] A comparison to the lifetime of liposomal colloidal dispersions in blood provides a useful benchmark.
against which to assess the circulation lifetime of other colloidal particles. The pharmacokinetics and biodistribution of colloidal particles are becoming the focus of increasing attention.[174, 177, 178]

One of the principal advantages of loading a drug into a colloidal particle, particularly for hydrophilic drugs, is that removal from the blood intact through filtration by the kidneys is drastically reduced. The cut-off for renal exclusion has been quoted to be approximately 5.5 nm.[179] Hydrophobic drugs are typically metabolised in the liver prior to excretion in bile. Drugs loaded into colloidal particles may also be protected from this elimination pathway.

A recent development has shown that high levels of PEGylation can be achieved on a particle surface by the creation of supported monolayers.[180] This particle consists of lipid coated to the surface of an inorganic acid sensitive nanoparticle. This supported bilayer can accept a much higher loading of PEGylated lipid than traditional liposomes and should therefore be more effective at creating a stealth corona.[181, 182] Mechanisms by which the stealth corona can be shed on response to a trigger are being increasingly investigated to improve the specificity and responsiveness of the colloidal particles.[183] For example in a series of papers Thompson and co-workers demonstrated the use of liposomes coated with an acid-labile polyethylene oxide layer. The removal of the PEG layer from the liposome surface, due to acid-catalysed hydrolysis, yielded controlled release of the cargo as the liposomes were destabilised. [184, 185]

The protection of the colloidal particle in an in vivo setting is perhaps one of the most crucial aspects in the development of advanced multifunctional nanoparticles, but it should be noted that it is only the first step towards the development of real-world nanomedicinal colloidal particles. Existing medical regulations will apply to nanoparticles including the nanostructured colloidal particles presented here and it is likely that a new regulatory pathway will emerge to assess the safety and toxicology of nanomedicines. Comprehensive assessments of the methodologies that could be used to evaluate nanoparticle immunotoxicity have been provided by Dobrovolskaia and co-workers [186-189]
The use of cytotoxic drugs in the chemotherapeutic treatment of cancer often leads to significant side effects and the non-specific delivery necessitates high dosages to achieve therapeutically-relevant concentrations in target tissues. The side effects of non-specific delivery are especially apparent in off-target rapidly dividing cells such as those of hair, bone marrow or the gastrointestinal tract. Targeting drug to a tumour lesion would clearly have significant effects in the reduction of the systemic dose, and thus hopefully the side effects. To our knowledge there has been no work published that assesses the targeting ability of cubosomes or other nanostructured colloidal dispersions although, theoretically, introducing this functionality is possible.

The first element inherent to colloidal particles that confers targeting ability is their size. The idealised diameter of colloidal particles is thought to lie between 10 to 150 nm. This size regime is realistic for cubosomes or hexosomes. The lower size boundary is well defined as it is the minimum size required to avoid rapid clearance through the kidney glomerula, this is estimated to be 5 to 6 nm for globular proteins.[179] It is harder to specify an upper size boundary. Colloidal particles have been shown to accumulate in mouse models in tumour tissue following systemic administration. This is attributed to the “Enhanced Permeation and Retention (EPR)” effect, a form of passive targeting.[190-193] EPR is relevant for tumor tissue due to the poorly formed leaky vasculature and compromised or absent lymphatic drainage in tumour tissue. Typical tumour angiogenesis results in fenestrated vasculature caused by aberrations in the basement membrane of the vessel. These gaps, which can be several 100 nm in size, lead to increased permeation of colloidal particles and increased retention time within the tumour tissue. However once sequestered within the tumor, extracellular diffusion may be more difficult due to the particle’s large diameter.[166]

Active targeting of particles through conjugation of specific targeting moieties such as antibodies or folate groups to the surface of the particle is thought to aid in both the localisation and the penetration of particles within tumour cells.[194] Several factors need to be taken into account when creating the targeting layer of the particle. These include the configuration of the antibody, where and how it is attached to the particle, and importantly the nature, orientation and size of the selected targeting moiety. Other strategies are highlighted in the review by Byrne.
et al. these include targeting using domains, and targeting of the up-regulated human epidermal receptors on cancer cell surfaces.[195]

Due to the multi-dimensional nature of the particles’ nanostructure and their internal water channels, it is possible for the targeting moieties, particularly small functional groups, to diffuse into the particles. The effect of this would be two reduce the effectiveness of the targeting domains. Anchoring to the surface has two main advantages: the first is that the internal structure of the particle is not altered and the second that all of the often expensive targeting moiety is available for binding.

There are three standard strategies commonly exploited in the covalent attachment of targeting molecules: formation of an amide bond, disulfide bonds or thioether linkages.[196] A comprehensive review of several chemical pathways has been provided by Nobs et al.[197] One of the preferred pathways for antibody fragment attachment is through selectively-reduced disulfide bridges of cysteine, as the resulting thiol groups typically ensure correct orientation of the antibody with no chemical modification required. A common strategy to link reactive groups with such antibody fragments is through the use of maleimide.[198] The reactive thiol groups on the antibody are typically located sufficiently far from the recognition domain that specificity to the antigen is retained. The attachment orientation of some ligands, and particularly antibodies or antibody fragments is crucial to their efficacy. Only correctly oriented targeting moieties can attach to specific ligands that are expressed or over-expressed at the site of lesion.

To ensure accessibility for specific binding, the targeting ligand is often attached at the end of a flexible functionalised PEO chain that acts a “spacer” moiety between the particle surface and the antibody. The use of a long spacer can increase the accessibility of the targeting group to the desired site by increasing the distance to the particle thus reducing the steric hindrance of the colloidal particle itself.

Preparation procedures are also important as clearly a top-down preparation technique such as ultrasonication with an antibody fragment already present will rapidly denature or degrade the targeting moiety. Covalent attachment of the targeting group to pre-dispersed particles, or incorporation into particles of a targeting group attached to a lipidic moiety are realistic approaches. The latter has been used with success for liposomes with PEG-lipid being successfully incorporated into the liposomes. This approach should be directly adaptable to colloidal systems of other lyotropic liquid crystalline phases.[199] Methodologies for antibody attachment to more complex
ordered nanostructures can be evolved from current technologies used for liposomes, and thus attachment of the antibody can take place at the surface of the particles or at the PEO terminus.[200]

A further advantage of drug delivery and imaging using colloidal particles, and specifically more complex particles such as cubosomes, is that the particles can be functionalised with multiple copies of the same ligands, or with multiple different ligands to improve attachment to the cell or lesion of interest. It should also be taken into consideration that the presence of large proteins on the particle surface may result in a greater immune response due to exposed non-pegylated surfaces and rapid clearance from circulation may therefore occur. Once at the site of interest, internalisation of the particles is a crucial step for effective therapeutic treatment.[201]
Conclusion

Advanced drug delivery vehicles, in order to be successful, need to demonstrate not only improved efficacy but also a reduction in side effects to make their adoption more widespread. Once the therapeutic elements have matured by demonstrating reliable performance, the imaging aspect of these particles can be extremely valuable not only as a diagnostic tool but also to track the whole dose administered. Additionally targeted drug delivery vehicles that can be spatially located in vivo would be very beneficial in the treatment of metastasised tumours to track any potential new lesions. Exploiting multifunctionality in colloidal lyotropic liquid crystalline drug delivery and medical imaging systems is a live possibility and harnessing the potential of each element is anticipated to progress rapidly once regulatory & safety concerns are addressed.

The outlook for lyotropic liquid crystalline colloidal dispersions in drug delivery/medical imaging systems is therefore positive although important research is required. Whilst significant research has been done on the creation of bioactive loaded colloidal lyotropic liquid crystalline particles, the translation remains in its infancy compared to other nanoparticle technologies.[202] There are still significant hurdles that must be overcome prior to their introduction to a clinical environment; continuous development.

These key elements that need to be addressed to ensure the successful progression of such particles to the clinic include shelf-life/stability, administration route and how to create made-to-measure therapies. The formation of stable dispersible self-assembled system is governed by the compatibility of the various bioactives, targeting, stabilising or medical imaging agents and, if any, the carrier matrix. The majority of these moieties are surface active and will thus affect the phase behaviour of self-assembled structures and their relative stabilities. It is therefore difficult to translate the success of one drug carrier to another bioactive based solely on the results obtained from the original bioactive. This can hinder the progress of self-assembled liquid crystalline dispersions as each novel system will have to be tested, potentially preventing the development of a one size fits all applications particle.[203] This issue has driven the introduction of high-throughput and combinatorial approaches to investigating compositional space in lyotropic liquid crystalline systems.[17, 19, 25, 54, 60, 98, 204, 205]

Another consideration which requires further research is the route of administration of this next generation of nanomedicines. A preferential route of administration is the oral pathway with which advanced antibody-based targeting technology is in incompatible. Other hydrolysable therapeutic moieties such proteins, peptides, RNA, DNA
may not be administered orally in all cases. Using advanced formulation and paying careful consideration to residence times it is possible to develop advanced formulations that can control time of release and release rates as well as protecting cargo from the surrounding environment.[117] Systemic drug delivery through intra-venous injection is a feasible route of administration for advanced drug delivery systems particularly in the treatment of potentially fatal diseases such as cancer. These drug delivery systems have to be able to cope with the immune response that they generate, which is typically achieved through the development of a stealth layer around the particle as extensively described herein.

In the development of nanomedicine, it is important to tailor the properties of the particle to the nature of the lesion of interest. For example, if a target is a life-threatening glioblastoma which can be treated by injection directly at the lesion site does a complex stealth corona need to be developed and optimised or should the focus be placed on active-targeting and effectiveness? For long term therapies or treatments that require multiple dosages, other administration routes obviously need to be suitable to the patient and to the nature of the particle. Whilst some studies have looked into circulation lifetime of the colloids little work exists on the effect of multiple systemic administrations and the concomitant effect this will have on the generated immune response.

Exploiting the non-covalent forces that creates the colloidal particles means the complex synthetic pathways that would be required to synthesise the equivalent polymeric or small-molecule materials can be bypassed. Using self-assembly as the particles’ framework means that dispersions can be created from elements of libraries that include the relevant therapeutic, medical imaging and targeting moieties. To exploit fully these libraries, the development of models that can predict the stability and nanostructure of multifunctional colloidal particles will play an important role in the future development of these dispersions. The potential of creating made-to-measure particles renders optimal therapies and diagnostic tools tailored to an individual disease scenario or even the specific patient possible.
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