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Amphiphilic designer nano-carriers for controlled release, from drug delivery to diagnostics

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Abstract

Vesicles formed by self-assembly of lipids and surfactants are increasingly recognised as carriers for drug delivery applications in disease targeting and many other biomedical-related areas, demonstrable by the growing number of significant publications. This manuscript reviews important facets of lipid-based vesicles as drug carriers and their surface modification to achieve controlled release and selective cell targeting. We cover both the more commonly used ionic phospholipid vesicle carriers and the rapidly growing field of non-ionic vesicles/niosomes using self-assembly of uncharged amphiphilic molecules, which could be formed using sugar surfactants or glycolipids, sorbitan esters, and polyoxyethylene alkyl ethers. Due to their lower cost, biodegradability, low-toxicity, low-immunogenicity, and specific sugar-cell recognition, much attention would be devoted to glycolipid bio-surfactants as potential carriers for targeted delivery. Specifically, our review points to the design consideration of lipid and surfactant nano-carriers based on critical packing parameter, membrane curvature, and the effects of hydrophobic chain structures. We also dedicate a section of this review to summarise some novel application of various lipid liquid crystal phases in drug delivery, and how in turn these are related to chemical structures of the lipid entities. The final section of this review outlines the application of lipid vesicles as delivery agents for diagnostic imaging.

Keywords: Target delivery, Sugar surfactants, Glycolipids, Lyotropic, Liposomes, Niosomes, Cubosomes, Hexosomes

1. Introduction

The era of nanomedicine has seen rapid advancement in drug delivery technology supported by the availability of a wide range of materials, improved physical understanding and techniques. To appreciate the scale of this development, we begin by reviewing a range of fashionable lipid vesicle carrier using phospholipid followed by its research progression into surface modification strategies for specific targeting. The past several decades have seen a remarkable increase in the interests for vehicles as carriers of bioactive agents at the nano-scale.[1] These nano-carriers can be used to encapsulate and deliver various compounds including, but not limited to enzymes, drugs, toxins, genetic materials, pesticides, nutraceuticals, and dyes.[2] Amongst the most popular and promising nano-carriers are liposomes, small vesicles composed of amphiphilic phospholipids.[3] Phospholipid consists of phosphate-bearing hydrophilic head group linked to hydrophobic fatty acid chains.[4] Formation of liposomal membrane bilayers in aqueous solution is mainly driven by hydrophobic interaction between the phospholipid fatty acid tails.[5] Their ability to entrap bioactive multiple molecules or cargo of both hydrophobic and hydrophilic nature and shield them from degradation, biocompatibility, biodegradability, and relatively low immunogenicity, have made liposomes a well-recognised drug and gene delivery carriers.[6] Advantages and disadvantages of liposomes and other types of nano-carriers are summarised in Table 1.[7-11]

Table 1 Lists of nano-carrier examples used commonly these days with their advantages and disadvantages

Delivery system	Feature	Advantages	Disadvantages	Ref.
Micelle	Single layer lipid	Ease of preparation, small size thus effective tumour passive targeting	Low loading capacity, poor *in vivo* stability	11
Liposome	Bilayer phospholipid vesicle	Natural amphiphile, biodegradable, biocompatible, able to encapsulate hydrophobic and hydrophilic molecules	Variable phospholipid purity, expensive, susceptible to oxidation which could induce cell toxicity, rapid *in vivo* clearance, surface modification required to improve stealth	10
Niosome	Bilayer non-ionic surfactant vesicle	Able to encapsulate hydrophobic and hydrophilic molecules, cheaper than phospholipids, chemically more stable than liposome, ease of storage and handling	Vesicle aggregation and fusion	10
Nano-emulsion	Nano-scale droplets of one immiscible liquid dispersed within another	Ease of preparation, thermodynamically stable	Requires high surfactant concentration during formulation	8
Cubosome	Cubic phase liquid crystalline nanoparticle	Tortuous structure, large surface area, high drug loading capacity	Complex, difficult to prepare, controlled release challenging, low encapsulation of hydrophilic cargo	7
Dendrimer	Highly branched polymeric nanoparticle	Controllable size, morphology, and functionality, low polydispersity	Could be cytotoxic due to the positive charge	9

Liposome carriers of different types (Figure 1) such as multilamellar vesicles/MLV, small unilamellar vesicles/SUV, large unilamellar vesicles/LUV, multi-vesicular liposomes/MVL, and sizes from nm to μm in diameters can be prepared depending on the compositions and techniques of preparation.[12] Bangham method or thin film hydration technique is one of the most popular ways to prepare vesicles, and is performed by dissolving lipids in organic solvent followed by solvent evaporation to form dry lipid film. The dry lipid film was then hydrated in aqueous solution and heated to temperature above its phase transition to form aqueous dispersion of multilamellar vesicles, which could subsequently be sonicated or extruded to form LUV and SUV vesicles.[13] SUV that has ~20-100 nm in diameter is thermodynamically unstable, tend to aggregate, has limited encapsulation ability, but has longer biological half-life than LUV (>100 nm).[14] Meanwhile, MLV, i.e. liposomes >0.5 μm diameter that contain "onion-like" multiple concentric aqueous chambers, appear to be more stable and better suited for carrying lipophilic cargo.[15] Unlike concentric MLV, multivesicular liposomes (MVL) has non-concentric multi-compartments, and been shown to exhibit higher loading capacity and sustained release compared to the multilamellar structures. However, the use of MLV and MVL in many drug delivery and therapeutics applications is still limited due to poor reproducibility and their heterogeneity in size and shape.[1]

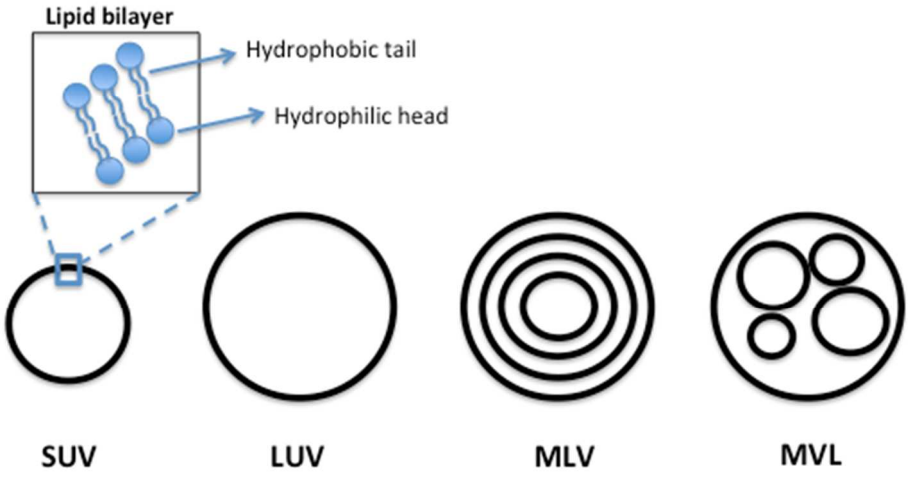

Figure 1 Schematic diagram of different types of liposomal vesicles: SUV (small unilamellar vesicles), LUV (large unilamellar vesicles), MLV (multilamellar vesicles), and MVL (multivesicular liposomes). Each circle represents a lipid bilayer structure (adapted from[16])

Conventional phospholipid vesicles/liposomes are composed of phosphatidylcholine (PC, a neutral phospholipid that contains fatty acyl chains) and cholesterol.[17] Incorporation of cholesterol into phospholipid liposomes has been shown to increase membrane stability and rigidity, as the rigid cholesterol molecules orient themselves such that the polar hydroxyl group directed toward the aqueous outer membrane while the hydrophobic rings in the bilayer; which reduces the movement of the fatty acyl chains

of the phospholipid.[18] Because no further surface modification follows, conventional liposomes administered through parenteral route, mainly intravenously, tend to have short plasma half-life and are rapidly taken up by macrophages. This was caused by the binding of liposomes with antibodies in blood serum/opsonins, which in turn attach to receptors on macrophages and result in endocytosis and clearance.[19] In addition, liposomes in blood stream are also able to interact, exchange lipids, and fuse with lipoproteins, where the entrapped active molecules would be released without reaching the target cells. [20]

Uptake of vesicles by macrophage was also correlated to the charge of the liposomes. Positively charged liposomes are generally regarded to be more cytotoxic compared to neutral and anionic liposomes. They also tend to have shorter half-life in blood serum compared to neutral and anionic liposomes due to higher non-specific serum protein interactions.[21] However, cationic liposomes were found to have a preferential uptake in tumour cells compared to anionic and neutral liposomes, and were retained in tumour tissues for a longer time.[22] Nevertheless, cationic liposomes are more often used for delivery of negatively charged nucleic acids in gene therapy.[23]

Essentially, an ideal carrier should be non-toxic, produced with high reproducibility, easy to scale-up, do not self-aggregate, high cargo loading, easily tunable to bind specific targets, no non-specific interactions, and have good biological half-life. Conventional liposomes are however far from ideal as they can suffer from short biological half-life, limited cargo loading, difficult-to-control cargo release, and binds non-specifically to biomolecules.[24] Depending on the saturation level of the fatty acid chains, phospholipid liposomes (particularly naturally derived phospholipids as they contain significant amount of poly unsaturation) can be susceptible to oxidation and cause structure breakdown.[25] Ways to improve the stability of liposomes and to prolong circulation time in drug delivery have been regularly demonstrated through liposomal modification as will be discussed in the next several sections. The role of carbohydrates as additives in liposomal formulation, which consist of single or mixed phospholipids with cholesterol, to enhance vesicle stability and specific cell targeting for controlled drug delivery, is discussed. We will also cover the fast growing glycolipid-based carriers field of research as there have been keen interests directed towards the development of vesicles synthesised from the self-assembly of uncharged amphiphilic molecules using sugar surfactants, sorbitan esters, and polyoxyethylene alkyl ethers. Similar to liposomes, these non-ionic vesicles/niosomes could be used to carry both hydrophilic and hydrophobic cargo for controlled release, while benefiting from lower cost and ease of handling due to higher chemical stability. The final section of this review describes the feasibility of vesicles and liposomes in diagnostic imaging, of which many works are still in progress.

2. Surface-modified liposomes

Surface modification is required to obtain desired chemical functionalities and to control how nano-carriers behave in biological environment as it influences the carrier interaction with surrounding molecules such as proteins, cells, and metabolites through various forms of interactions. Adsorption of these biomolecules could potentially alter the properties of the vesicles such as charge and conformation, and, being recognised as foreign objects, would trigger the immune response that resulted in clearance. Therefore, strategies are needed to "trick" the immune system into believing that the vesicles belong to part of the body, and shield them from non-specific biomolecules interaction.

2a. Stealth liposome

It has been widely accepted that modification of phospholipid liposomes with non-ionic bio-surfactants or polymers was one of the most successful approaches to improve stealth, i.e. prolonged liposome circulation time in blood plasma.[26] A gold standard approach is to incorporate hydrophilic poly(ethylene glycol)/PEG (also known as polyethylene oxide/PEO or polyoxyethylene/POE depending on the molecular weight) into liposome to create a steric barrier against macrophage uptake.[27]

PEG ($H(OCH_2CH_2)_nOH$, molecular weight below 20 kDa is an FDA-approved neutral, non-toxic, and biocompatible polymer that is well known for its ability to reduce bioadhesion.[28] This polymer is highly mobile in aqueous solution with large exclusion volume, and has a strong tendency to form hydrogen bonds with water and provide a physical barrier from the adsorption of biomolecules, which could be characterised by examining the fixed aqueous layer thickness/FALT.[29] Incorporation of PEG into liposomes is commonly carried out by adding PEGylated lipid derivatives (commercially purchased or in-house produced by covalent grafting of PEG to a hydrophobic anchor) into liposome constituents during formulation.[30] Hydrophobic anchor generally used to couple PEG is phosphatidylethanolamine (PE) due to its reactive amines. These amine groups could be coupled to hydroxyl end of PEG molecule to form stable urethane linkages;[31] or react with succinic anhydride-modified PEG to form amide bonds;[32] or coupled to NHS-modified PEG.[33] Different types of anchors with varying chain lengths, as well as characteristics of the PEG polymer such as molecular weight, have been shown to affect liposome stability in blood plasma and drug delivery efficacy.[34] Higher PEG molecular weight was shown to have larger FALT, which generally results in longer blood circulation time and higher drug concentration in tumour and plasma.[35] On the other hand, shorter PEG chain length was shown to be rapidly cleared from blood circulation through liver and spleen.[36]

In traditional non-surface-modified PEG liposome systems, liposome-encapsulated active agents are usually delivered to the tumour sites through passive targeting.[37] Unlike normal blood vessels which are lined by endothelial cells with continuous tight gaps of about 2 nm, newly formed blood vessels which supply nutrients and oxygen to tumours are filled with 'leaky' gaps between the endothelial cells that can span from 100 to 600 nm due to accelerated growth. This allows tumour cells to enter the bloodstream and spread towards other parts of the body.[38] Therefore, anti-cancer drugs encapsulated in small sized liposomes delivered intravenously could enter the tumour sites through this

leaky vasculature and absorbed by the cancer cells as shown in Figure 2. This delivery phenomenon is known as 'passive targeting'.[38, 39]

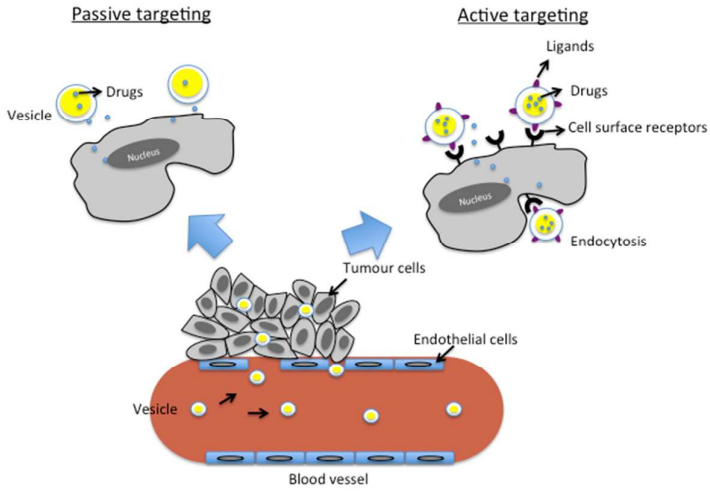

Figure 2 Schematic representations of the passive (left) and active (right) drug-tumour targeting. In passive delivery, vesicular nanoparticles extravasate through leaky vasculature, accumulate, and release the encapsulated drugs; whereas in active targeting delivery, ligands-attached vesicles extravasated through leaky vasculature specifically bind to the cancer surface cell receptors followed by receptor-mediated endocytosis. Drugs could be released either extracellular or intracellular through endocytosis (adapted from[40]).

Successful implementation of this technique is therefore highly dependent on the type and site of tumours, vascularisation, and assuming that all the liposomes is taken up through enhanced permeability and retention (EPR) effect with no prior clearance.[38] Although small sized liposomes (typically 200 nm or less) extravasate to tumour cells via EPR effects and are typically used for disease tumour targeting,[41] Kibria *et al*. recently showed that large size liposomes of ~ 300 nm diameter size, if modified with ligands, could have better therapeutic efficiencies than ligand-attached 100 nm liposomes.[42] Attachment of ligands to the outer surface of liposomes that are able to bind specifically to target receptors at certain sites such as cell surface or extracellular matrix of cells is known as 'active targeting'.[39, 43] Modification of the liposome surface is therefore vital as it controls the binding behaviour between liposomes and ligands, which subsequently affect ligand-receptor interaction, liposomal stability, and its interaction with biomolecules.[44]

As proteins are macromolecules that consist of hydrophobic, uncharged and charged hydrophilic surfaces, they interact almost immediately with vesicles or liposomes through one or more interactions including van der Waals, hydrogen bond, hydrophobic, and electrostatic forces. These interactions could cause protein conformational changes that result in the loss of protein function and activities, liposomal dissociation and unwanted drug leakage.[45] Release of entrapped drugs through liposome membrane diffusion was also reported to be concentration dependent where an increase in drug release rate constant was generally observed at diluted liposome suspensions[46].

Besides PEG polymers, non-ionic surfactants such as POE-lipid conjugate which contain ethylene oxide head group linked to hydrophobic alkyl chain tails has been investigated for their stealth properties and drug delivery applications.[47] Other non-ionic surfactants that have shown an increased circulation time are molecules that contain sugar head group due to the presence of -OH moieties, which are capable of binding water molecules through hydrogen bonding.[48] The use of sugar surfactants and sugar-conjugated liposomes in drug delivery will be discussed in the section 2c and 3 as they provide not only stealth properties but are also capable of specific cell targeting.

2b. Targeted liposomes: ligand conjugation

In tumour targeting, liposomes can be modified with antibodies or their fragments that target tumour-specific antigens;[49] or with ligands that bind to upregulated receptors present on tumour cells.[50] These ligands and antibodies can be conjugated to functional groups in liposomes; or PEGylated liposomes that contain functional end groups (Figure 3). In this case, liposomes were synthesized with homo-(eg. N-hydroxysuccinimide(NHS)-PEG$_n$-NHS) or hetero- (eg. NHS-PEG$_n$-maleimide, NHS-PEG-aldehyde) bifunctional PEG linkers where phospholipid was bound to one end, while leaving the other functional PEG end available for conjugation with ligands and biomolecules. [51]

Liposomes that carry antibodies or their fragments on the outer surface (immunoliposomes) could be used to detect tumour in its early stage, deliver the entrapped anti-cancer drug to the tumour site, induce cell apoptosis, block growth factor receptors, block angiogenesis, or destroys the tumour cells through a process known as antigen-dependent cellular cytotoxicity.[52] An antibody molecule is composed of two glycosylated heavy chains of large polypeptides linked by disulfide bonds to light chains of smaller polypeptides.[53] Therefore, functional sites of the antibodies that are commonly used for conjugation or labeling are primary amines (-NH_2) from the abundant lysine residues and the N-terminus of the polypeptide chains, carbohydrates from glycosylated Fc region, and the sulfhydryl group (-SH) from cysteine residues of the polypeptide chain or reduction of the disulfide bonds (S-S) at the hinge region.[54]

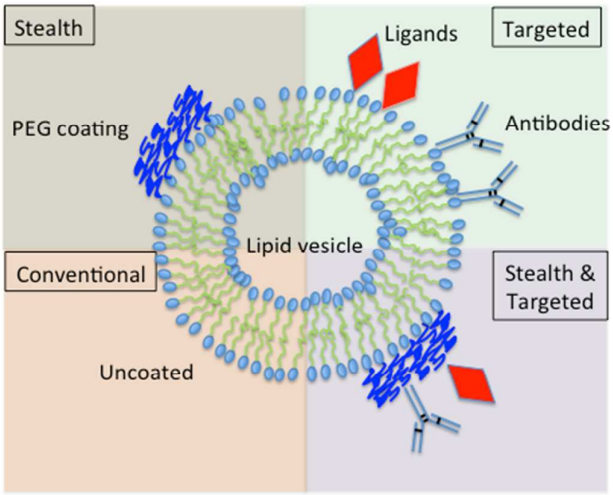

Figure 3 Schematic representation of a conventional and modified liposome

Conjugation of amine groups in antibodies to modified- or non-modified liposomes can be carried out using 1-3-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) reaction chemistry to conjugate amines to phosphates in liposomes.[54] Other derivatives that can be used to bind amines are aldehyde-containing liposomes such as oxidized glycolipid-liposomes to form unstable Schiff base intermediates that could be reduced using sodium cyanoborohydride;[54] liposomes that contain epoxy groups for reaction with primary amines to form secondary amine bond;[54, 55] and maleimide-modified liposomes.[54, 56] These maleimide groups can also be used to covalently react with sulfhydryl groups of the antibodies or ligands through stable thioether bonds.[57]

Meanwhile, -OH residues of carbohydrates in glycosylated antibodies and ligands can be coupled to liposomes through epoxy-modified liposomes to form stable ether bonds;[54, 55] amine-or hydrazide-modified liposomes through oxidization of hydroxyl groups with sodium periodate to produce reactive aldehydes;[54] isocyanate-modified liposomes to form carbamate linkage;[54]or aminated-liposomes to sugar hydroxyls that have been acylated using N,N-disuccinimidyl carbonate reagent.[54] The conjugation processes described could also be applicable to proteins, peptides, or other ligands that carry specific functional groups. Although ligands can be tethered to liposomes in a stable manner, one should also assess changes in overall liposome characteristics such as conformation, size, charge, stability, and bioactivity caused by conjugation.

A current research highlight entails multiple-conjugation of liposomes where the liposomes are modified to carry different types of ligands. Dual-targeting liposome developed in the past few years was primarily used to enhance targeting selectivity for therapeutic efficacy.[58] Additionally, some tumour cells can express various types of receptors such as human KB cell line which has both folic acid receptor and epidermal growth factor receptor/EGFR.[58] These receptors could be targeted using liposomes conjugated with both folic acid and EGFR-antibody. In some studies, multiple types of antibodies were conjugated to drug-loaded liposomes to target different antigens on tumour cells, where authors observed an improved selective toxicity of anticancer drugs towards tumour cells.[59]

2c. Targeted liposomes: carbohydrate-conjugation

Carbohydrates play important roles in various cellular processes including cell recognition, adhesion, and growth;[60] and are present on cell and virus surfaces.[61] One development trend in liposome modification is to incorporate sugar molecules into phospholipid liposomes for targeted drug deliveries.[62] This can be achieved through attachment of carbohydrate moieties to phospholipid liposome surface; or through addition of glycolipids to phospholipid liposomes during fabrication. Grafting of different types of carbohydrates to liposomes have been used to target different disease sites while, due to water-sugar hydroxyl binding, minimize their non-specific interactions with other biomolecules, prolonged plasma circulation, reduced macrophage uptake and improved stealth.[62]

Hyaluronic acid (HA, a non-sulfated glycosaminoglycan), for example, is known to bind specifically to CD44 surface glycoprotein receptor that is overexpressed in various cancer cells; and

thus has often been used to modify phospholipid liposomes.[63] The carboxyl group of the HA molecules can be activated with EDC which then bind to the amine group in phospholipids such as PE and phosphatidylserine (PS). Alternatively, PE-HA conjugate can be formed through reductive amination of the aldehyde–functionalised HA (formed through oxidation of hydroxyl groups using sodium periodate) to amines in PE using sodium cyanoborohydride reducing agent. These strategies can also be used to bind sugars to macromolecules that contain amine such as proteins, enzymes and antibodies to form sugar-macromolecule conjugates useful for macromolecular glycotargeting carrier system. Delivery of drugs using macromolecules is not the scope of this review, but the following reference[64] could serve useful for interested readers.

Other examples of sugar specific targeting are binding of mannosylated liposomes to mannose receptors at liver non-parenchymal cells;[65, 66] galactosylated liposomes to asialoglycoprotein receptors in liver parenchymal cells;[66, 67] recognition of human breast cancer cells-expressed N-acetylgalactosamines using lectin;[68] over-expressed transferrin receptors at various cancers using transferrin glycoprotein-liposome;[69] p-selectin receptors on tumour cells using p-selectin glycoprotein ligand;[70] tumour-expressed E-selectin using Sialyl-lewis X oligosaccharide-liposome;[71] and glycolipid trehalose dibehenate - cationic liposomes to bind to Mincle ligand to induce cell-mediated immune response.[72]

Inclusion of glycolipids into phospholipids could alter the overall liposomal bilayer characteristics including charge, stability, and phase transition behaviours.[73] Sekiguchi *et al*. reported that at a certain amount, addition of glyceroglycolipids (mono-and di-galactosyl glyceride) to phospholipids would stabilize the bilayers although destabilizing effect was seen at high glycolipid concentration. Moreover, an increased overall liposome particle size and decreased zeta potential (less overall charge) was observed.[74] They could also serve to decrease the tendency of unilamellar phospholipid vesicles to aggregate at below the gel-liquid crystalline phase transition temperature, as reported by [75].

3. Conventional non-ionic vesicles

Owing to their amphiphilic nature, sugar-based surfactants and glycolipids can also be used as starting materials to form vesicles.[76-78] A sugar amphiphile consists of hydrophilic sugar units bond to one or more hydrophobic tails and can be naturally or synthetically derived. The sugar head moieties can be monosaccharide (mannose, glucose, galactose), disaccharide (lactose, maltose, sucrose, or trehalose), or polysaccharides.[79] Some of the naturally-derived sugar-based amphiphiles are bacterial biosurfactants such as rhamnolipids, sophorolipids, trehalose lipids, and mannosylerythriol lipids, and natural glycolipids including sphingoglycolipids (mainly from animal lipid extracts) and glyceroglycolipids (mainly from plant chloroplast and cyanobacterial lipid extracts). Examples of the synthetic sugar-derived non-ionics are the food-additive surfactants (Figure 4 A) such as sorbitan esters (Span), poly(oxyethylene) sorbitan esters (Tween), and sucrose esters; and the straight-chained glycosides (Figure 4B) such as alkyl glycosides and alkyl polyglycosides.[79-81]

The use of sugar-based surfactants or lipids, in particular synthetic ones, for alternative drug carriers to (phospholipid) liposomes is beginning to receive much attention in the recent years.[78, 82, 83]

Because of their low toxicity, biodegradability, and non-immunogenicity, these non-ionic sugar-based surfactants have been used in cosmetic, food, detergent, and pharmaceutical applications as emulsification, dispersion, and wetting agents.[84] At present however, application of sugar amphiphiles for targeted disease drug delivery such as tumor-targeted one is still in its infancy compared to phospholipid liposomes.

3a. Food-additive non-ionic surfactants

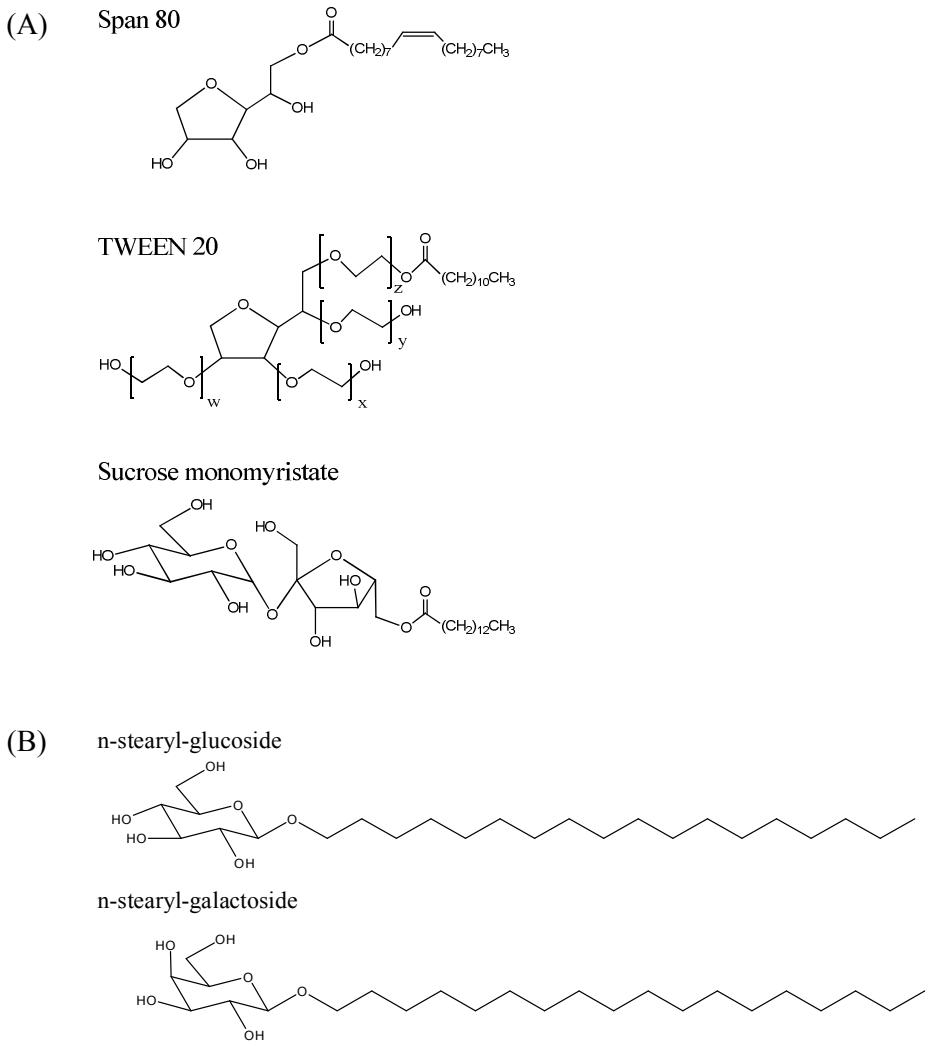

Figure 4 (A) Examples of conventional non-ionic surfactants. These commercial products are supplied as isomeric mixtures, and their homologues are accepted as food additives. (B) Examples of alkyl glucoside and galactoside possessing a straight chain.

Hydrophilic-lipophilic balance (HLB) of industrial surfactants is the empirical and practical index in applications such as emulsification, solubilisation, foaming and so on. Surfactants having a low HLB value (< 6) tend to be more soluble in organic solvents and prefer water/oil (W/O) emulsion, while a high HLB value (> 11) indicates a more hydrophilic compound. For bilayer formation, the surfactants with HLB of 7-14 (or around 10) are empirically desirable.[85, 86]

Sorbitan esters or Spans are fatty esters of sorbitan, the cyclized derives of a sugar alcohol sorbitol (Figure 4A). Although Span series surfactants are inexpensive and widely used as food additives, they are rather heterogeneous (they contain ten or more components) and have a low HLB number. Spans are essentially hydrophobic and mainly used for W/O emulsification. For higher hydrophilicity, their PEGylated derivatives TWEEN (HLB > 10) have been used for O/W emulsification or solubilisation. [87] Sucrose esters ("sugar esters") are fatty esters of sucrose and also widely used as food additive surfactants. Sugar esters with HLB 3 - 18 are commercially available.

Kato and co-workers evaluated and demonstrated the feasibility of a non-ionic surfactant, Span 80 (sorbitan monooleate), as novel drug delivery carrier.[88-90] Due to its too high hydrophobicity, commercial Span 80 (HLB 4.3) does not form stable bilayers by simple aqueous solution dispersion; and additives such as cholesterol or negatively charged dicetyl phosphate are often added. Therefore, a two-step emulsification method was adopted to partially purify and enrich the diesters to form unilamellar vesicles. The authors showed that the modified Span 80 vesicles have higher membrane fluidity than phospholipid liposomes, and can be used to carry a specific lectin to target mannose-rich human cancer colon cells with inclusion of PEGylated lipids to vesicles being suggested to exhibit higher anti-tumour activity than non-PEGylated Span vesicles.[91] Ability of other non-ionic surfactants Tween to form vesicles has also been studied.[92, 93] Tween surfactants are highly soluble in aqueous solution and tend to form lamellar structures instead of vesicles.[92, 94] It is generally accepted that increasing the size of neutral carbohydrate headgroups (i.e. higher HLB values) favours the formation of lamellar bilayers.[95] Vesicles with sugar surfaces have also been examined by mixing sugar esters with cholesterol and dicetyl phosphate.[82, 96]

3b. Straight-chained glycosides

Alkyl glycosides are biodegradable, non-toxic, and non-irritating non-ionic surfactants synthesised from renewable raw materials, and have received major interests as emulsifiers in cosmetics, food, and pharmaceutical applications. [80, 81] Depending on the properties, alkyl glycosides could also be used to form vesicles for drug delivery carriers, as has been described by Kiwada and co-workers in the 1980s.[97-99] They studied the formation of vesicles and encapsulation efficiencies of different types of alkyl glycosides with various alkyl chain lengths and sugar. The authors conclude that alkyl glycoside vesicles show better encapsulation efficiencies and higher stability in plasma solution than PC liposomes, although initial rapid cargo release was observed.[98]

The same authors also investigated tissue distribution of alkyl glycoside (stearyl galactoside and stearyl glucoside) vesicles on mice after intravenous administration. Both alkyl glycosides showed less spleen uptake compared to PC liposomes; but there was a preferential uptake of galactosylated alkyl glycoside vesicles in liver.[97] This shows that galactosylated vesicles could be utilised for hepatocyte-selective targeting. In another study, Daicho *et al*. investigated the effects of incorporating alkyl glycoside (n-dodecyl glucoside and n-dodecyl sucrose) to liposomes on *in vivo* tissue distribution. Their results showed that both alkyl glycosides, particularly sucrose-bearing alkyl glycosides, had low spleen and liver uptake and higher tumour tissue accumulation.[100] Using alkyl glucopyranosides surfactants formulated with cholesterol as vesicle stabiliser, Muzzalupo *et al.* recently showed high

entrapment efficiency of anticancer drug methotrexate with prolonged *in vitro* drug release.[101] In applications other than tumour drug delivery, alkyl glycoside vesicles could also be used to enhance drug skin penetration as has been recently showed by our group using maltosylated and lactosylated alkyl glycosides.[77]

3c Glycolipid vesicles: current and future directions

The past decade has seen a steady development in sugar-based vesicles for drug delivery applications, with much emphasis placed on the synthesis, design and formulation optimization, and physical characterization. Effects of vesicle properties on the entrapment efficiencies of different types of drugs, their *in vitro* release behaviours, and haemolytic activities are commonly investigated, and are intrinsically application-specific. Although the research of pharmacokinetic properties of the drug-entrapped vesicles is ongoing, much studies are still required to understand the absorption, distribution, metabolism and elimination of the drugs administered through different routes. Meanwhile, evaluation of the pharmacodynamic properties including vesicle and drug-receptor interactions as well as their effects on human body is also of utmost importance to infer the underlying physiological process with biological endpoint.

4. Design Consideration of Amphiphile-Self-Assembled Nanocarriers

In order to further develop novel nano-carriers based on surfactant molecular assemblies, we need to consider relationship between molecular detail structures and self-assembled structures or properties. This section describes three topics: how surfactants and lipids pack themselves, self-assembled structures based on lyotropic liquid crystals, and effects of hydrophobic chain structures on membrane integrity and impermeability. This can be a helpful guide to design self-assembled nano-carriers and tune their drug encapsulation efficiency and release control.

4.1 Critical packing parameters of surfactants and lipids

The molecular shape of an amphiphile (a surfactant or a lipid) influences the molecular packing in self-assemblies.[102-105] This leads to different structural and physical properties of the drug carriers hence drug entrapment efficiencies and release mechanisms. For describing the effective molecular shape, the critical packing parameter (CPP) of the amphiphile is defined as v/a_0l_c. Here, v is the volume of the lipophilic chain, a_0 is the interfacial area occupied by the hydrophilic head group, and l_c is the length of the lipophilic chain.[85] Estimating this parameter of a certain surfactant or lipid enables one to predict its molecular packing and preferred structure of spherical or cylindrical micelles, bilayers, vesicles, or inverted micelles.

In the case that a cone-shaped surfactant has a large hydrophilic head group with a short lipophilic chain and its CPP value is 1/3 or smaller, the surfactant favors the formation of spherical micelles (Figure 5). Cylindrical micelles are preferentially formed when the hydrophilic head group of a surfactant is somehow small compared with a single lipid chain and its CPP is between 1/3 and 1/2.

For a flexible bilayer or a vesicle, the CPP should lie between 1/2 and 1, which could be formed by a double chain lipid with a relatively large hydrophilic head group. When the shape of a surfactant resembles a cylinder with a double chain and a small head group where the CPP is close to 1, planar bilayers are preferentially formed. If a surfactant has a large lipophilic group with a small hydrophilic head and its CPP is larger than 1, inverted or reversed micelles would be observed.[106]

This paragraph gives some remarks on the CPP value, a theoretical parameter to predict a possible phase from a given structure. Firstly, this value is most sensitive to the effective hydrated interfacial area a_0, and the head group hydration amount drastically changes with the type of the molecular packing, which in turn is predicted from the CPP value (a kind of circular reasoning). Despite the challenging interdependency, the CPP can be a useful, semi-quantitative parameter. For example, a lyotropic liquid crystal can be analyzed for its structural dimensions and hydration amount, if its phase diagram (phase structure and composition boundary) is evaluated by various experimental techniques (or in combination) such as X-ray diffraction, NMR, and fluorescence spectroscopy.[107-111] If homologous amphiphiles are comparatively examined, using CPP one can discuss a (semi-) quantitative tendency of their molecular packing in relation to their hydrated headgroup cross-sectional area.[112] Secondly, it is useful only for fluid states. Sodium dodecyl sulfate (CPP = 0.36) in water forms a spherical micelle at 25 °C, but precipitates into a tilted-bilayer hydrated solid below 16 °C (its Krafft point, the melting point in water). Alternative to the CPP value, membrane curvature is more conveniently used for describing various normal to inversed lyotropic liquid-crystalline phases including cubic ones, as shown in Figure 6, section 4.2.

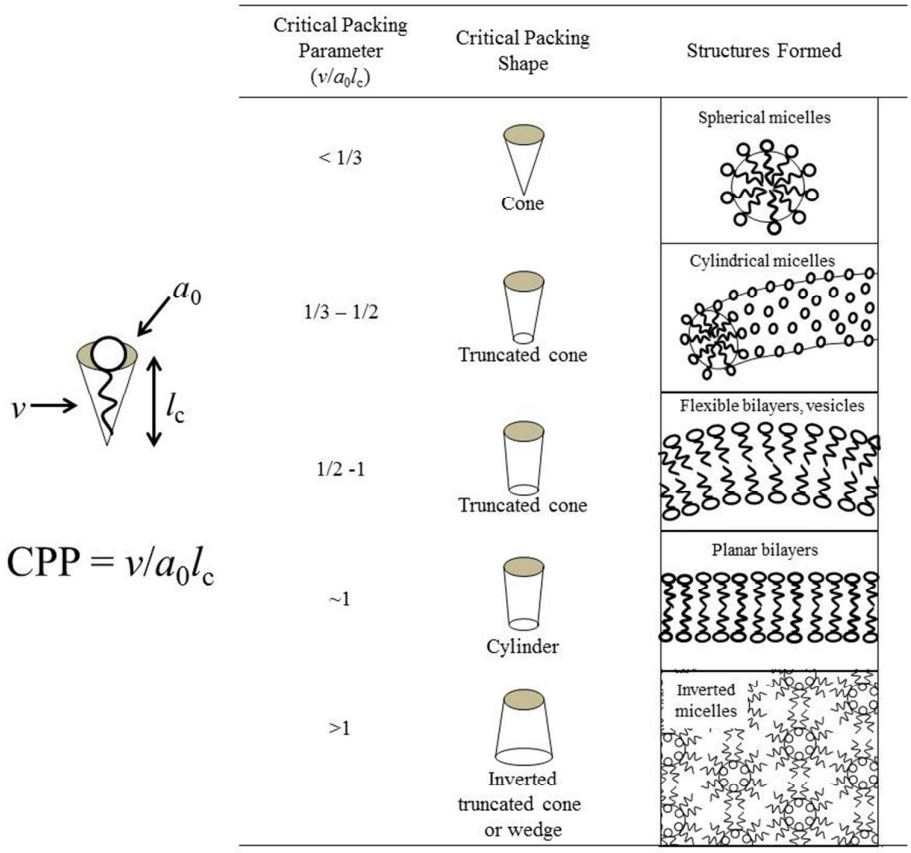

Figure 5. The molecular shapes and critical packing parameters of surfactants and lipids and the structures formed. This figure was redrawn from [85].

4.2 Membrane curvatures and exotic carriers based on lyotropic liquid crystals

Figure 6 exemplifies lyotropic liquid-crystalline phases found in amphiphile/water systems. For simplicity, only the phase types of the fully hydrated amphiphiles are mapped in a function of the membrane curvature. Membrane curvature was described to be positive when the hydrophilic group splays out toward the water region.[113] From the positive to negative mean curvature (the right to the left side across the diagram), the system shifts from the hydrophilic normal phases (a normal micellar solution, I_I, H_I, and Q_I) *via* the L_α phase to the hydrophobic inversed phases (Q_{II}, H_{II}, and I_{II}). Towards the far left and right positions, the normal micellar aqueous solution and the inversed micellar solution diluted with oil are located.

The planar lamellar phase (L_α) is a surfactant or lipid lyotropic liquid crystalline phase with its curvature around zero. The phase consists of stacks of ordered amphiphilic bilayer aggregates separated by thin aqueous layers. If the diluted phase can coexist with an excess aqueous phase, the dispersion may form a closed vesicle, where the bilayer membrane separates its inner aqueous content from the outer aqueous solution. Further, if the membrane acts as an effective barrier, the vesicle can be used for controlled drug release.[114, 115]

In the normal hexagonal phase (H_I), amphiphiles are arranged in cylindrical micelles ordered

on a hexagonal lattice with polar head groups directed towards outer aqueous phase.[102, 105] The normal bicontinuous cubic phase can appear in the location between an L_α phase and H_I phase and show an interwoven fluid porous structure made of lipid bilayers in a body-centered cubic lattice. The normal micellar cubic phase can appear between an H_I phase and a normal micellar phase, and its structure is based on various packing of spherical or slightly anisotropic micelles in cubic lattices. Dilution of these normal lyotropic phases results in formation of normal spherical or cylindrical micelles, which are effective for solubilisation of lipophilic materials in aqueous solutions and O/W emulsification.

In contrast, the inversed hexagonal (H_{II}), inversed bicontinuous cubic (Q_{II}) and inversed micellar cubic (I_{II}) phases are water-insoluble. Their hydrated hydrophilic moieties are internally located and surrounded with the hydrophobic chains. [116-118] They maintain their internal structures in excess aqueous solutions. Wrapped by amphiphilic block co-polymers such as Pluronic and Poloxamer, the Q_{II} and H_{II} phases are dispersible in excess aqueous solutions. The cubosomes (aqueous dispersed Q_{II}) and the hexosomes (aqueous dispersed H_{II}) in controlled drug delivery and release have been investigated in the two decades due to their unique structures that consist of aqueous channels separated or partitioned by lipid bilayers.[119, 120] For the inversed cubic and hexagonal phases, monoolein (MO, identical to glycerol monooleate GMO) has been most widely used although the ester can be hydrolysed. As chemically stable alternatives, phytantriol and phytanyl xyloside have been examined recently.[121] Examples of the different drug delivery applications in various liquid crystal phases are tabulated in Table 2.

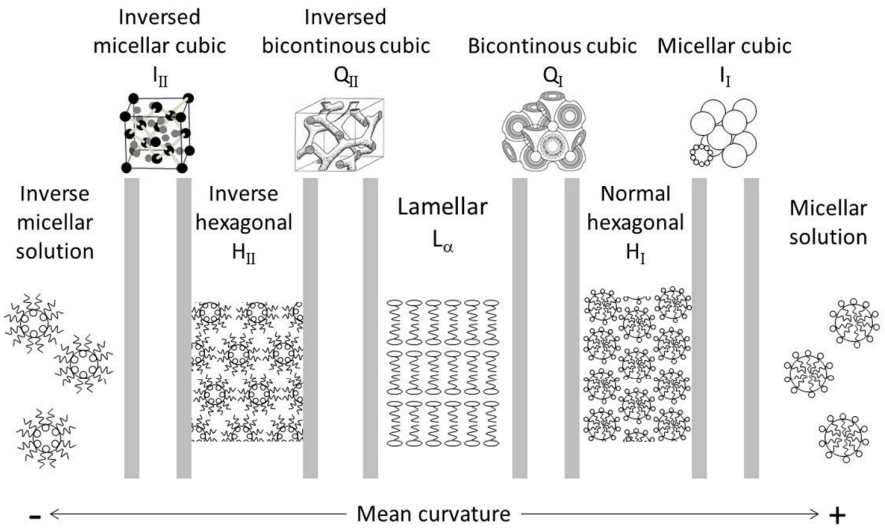

Figure 6. The sequence of lyotropic liquid crystalline phases, arranged according to their average membrane mean curvature. The curvature can change with composition, hydration, temperature, and pressure. This figure was based on a figure in [113] with additional illustration.

Table 2 Examples of liquid crystal phases and their potential applications.

LC phase	Formulation	Encapsulated material	Size (nm)	Route of administration	Potential application
Normal Micelle (L_1)	Lecithin/Cholate [122]	Diclofenac	-	Transdermal	Rheumatism,skin inflammation
	Glycolipid-like chitosan/Stearic acid[123]	Paclitaxel	40	Intravenous	Cancer
	Polyethylene glycol/PE[124]	Meso-tetraphenyl	6	Oral	Cancer
	Phospholipid/Cholate[125]	porphine Silybin	80	Oral	Liver disorders
Lamellar (L_α)	Sucrose ester[96]	Diclofenac	150-314	Transdermal	Skin inflammation
	Sucrose ester[96]	Sulfadiazine	218-335	Transdermal	Infected burn
	Phospholipids/Alkyl glucosylceramide[126]	Doxorubicin	110	Intravenous	Cancer
	Phospholipid mixtures[127]	Insulin	<250	Oral	Diabetes
Inversed Hexagonal (H_{II})	Monoolein/Oleic acid/Pluronic F-68[128]	Progesterone	250	Oromucosal	Hormone therapy
	Monoolein/Oleic acid/Poloxamer 407[129]	Cyclosporine A	182	Topical	Cutaneous diseases
Inversed Bicontinuous Cubic (Q_{II})	Phytantriol/Poloxamer 407[130]	Ibuprofen	240	Oral	Anti-inflammatory
	Glyceryl monooleate/Poloxamer 407[131]	Simvastatin	100-150	Oral	Bone growth
	Monoolein[132]	Minoxidil/Hydroxypropyl b-cyclodextrin	<400	Transdermal	Hair loss

4.3 How can we tune the glycolipid chain characteristics?

In addition to the total molecular shape (CPP) or membrane curvature, the molecular detail of hydrophobic part is a critical factor to the self-assembly characteristics of a glycolipidWe hereby discuss the effects of alkyl chain length and the unsaturation degree, lipophilic additives, and alkyl chain branching. The effects of sugar head group structures have been discussed elsewhere [103, 133]

The chain length and unsaturation degree strongly affects the lipid phase transition temperature T_m between the hydrated solid phase ("gel" phase) and the liquid crystalline phase. [134, 135] This can be measured using techniques such as differential scanning calorimetry, optical polarizing microscopy and phase fluorometry.[136] A longer and saturated chain results in a higher T_m of a hydrated lipid owing to tighter chain packing.[134, 137] A lipid having a shorter or unsaturated chain (most natural lipids) shows a lower T_m and exhibits higher membrane fluidity, permeability, and increased leakage.[138]

Span 80 with unsaturated oleoyl chain showed lowest entrapment efficiencies of water-soluble drugs compared to other Spans. Span 60 with saturated C16 chain (T_m ~ 55 °C) has higher encapsulation efficiencies than Span 40 and 20 having C14 and C12 chains.[139, 140]

Many groups have studied the entrapment efficiencies of hydrophilic, amphiphatic, and lipophilic drugs in several sugar surfactants.[77, 93, 140, 141] These works suggest a general tendency that higher encapsulation efficiency of hydrophilic substances (percentage of entrapped drug) was obtained when a surfactant system has a high T_m with a less leaky membrane. On the other hand, the high T_m can be a severe problem in encapsulation, particularly in encapsulation of temperature-sensitive drugs or proteins. Many encapsulation methods require a step above the phase transition temperature T_m. Although dialysis or phase inversion vesicle preparation can proceed around or below room temperature, dissolution with a detergent or an organic solvent is an inevitable step.[142] Two approaches to solve this problem are described in the following paragraphs in this section.

Addition of cholesterol to phospholipid liposomes remarkably affects the membrane fluidity and impermeability. Above the phospholipid T_m, cholesterol addition (typically 10 - 20%) decreases the membrane fluidity compared with the pristine liposome membrane. It is also noted that the nature of the encapsulated drugs can influence vesicle formation and stability. For instance, incorporation of hydrophobic drug such as anti-cancer Paclitaxel into nonionic sugar surfactant vesicles has been shown to improve stability of the niosomes, less drug leakage, and an improved transdermal delivery.[143] In encapsulation of Doxorubicin, an amphiphatic anti-cancer drug, Span 60 niosomes was shown to cause vesicle aggregation and steric stabilisers such as Solulan-24 (poly-24-oxyethylene cholesteryl ether) should be added.[48]

It is also noteworthy that chain branching can drastically decrease the lipid T_m and improve the membrane integrity and impermeability.[144] It has been reported that an isoprenoid ether chain, 1, 2-di-O-phytanylglycerol, is one of the major hydrophobic chains in the plasma membranes of archaebacteria extremophiles which survive in harsh environments such as at pH 3, at high salt concentrations, and in hot springs. The phytanyl-ether lipids play critical physiological roles in maintaining the membrane integrity under the environments. We have synthesised a model glycolipid Mal_3Phyt_2 from a branched isoprenoid alcohol phytanol, and examined the phase behavior and membrane impermeability of the lipid. The lipid in water exhibited an L_α lamellar liquid crystalline phase at room temperature and a bilayer vesicle was prepared in dilute dispersion at room temperature. For solutes such as ions and fluorescence dyes, the closed vesicle shows higher membrane impermeability than conventional phospholipid vesicles. The results demonstrate that the isoprenoid glycolipids can form reinforced fluid bilayer membranes.

We have shown that isoprenoid-chained glycolipids have low T_m and the dependence of different lyotropic liquid crystalline on molecular shapes.[103] The double-chained isoprenoid glycolipids exhibit a lamellar or inversed lyotropic phase only, while the single-chained isoprenoid glycolipids can form a micellar solution, a vesicle, or an inverted cubic structure. Owing to the chain branching, another type of branch-chained glycolipids "Guerbet-chained glycolipids" [103] also show low melting points despite of their long alkyl chains. The asymmetric branched chain Guerbet glycolipids promote the tendency to form non-lamellar structures. For construction of future efficient nano-carriers, we may

consider these branch-chained glycolipids as good candidates satisfying a low membrane melting point and high membrane impermeability simultaneously.

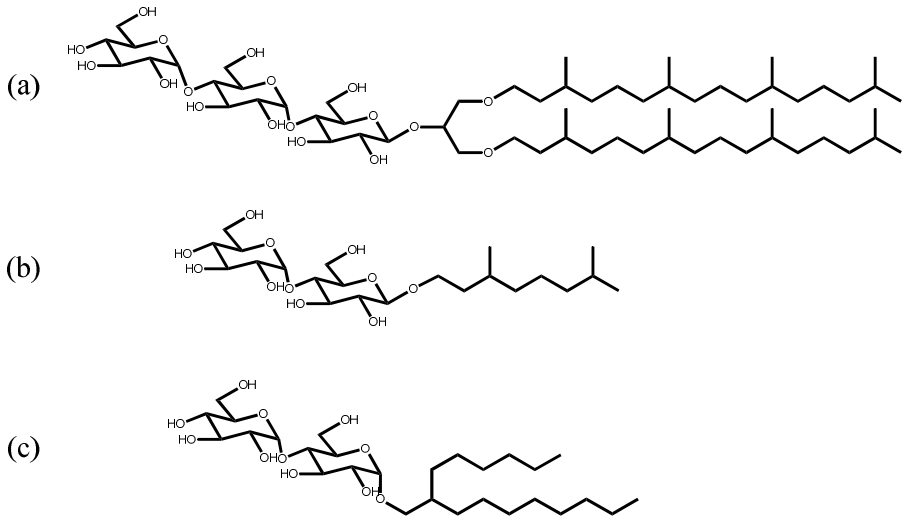

Figure 6. Examples of synthetic branch-chained glycolipids. (a) Double-chained isoporenoid glycolipid Mal_3Phyt_2. (b) A single-chained isoprenoid glycolipid. (c) A Guerbet-chained glycolipid.

5. Liquid crystal phases and routes of administration

Appropriate carrier system should be designed to cater for the different requirements needed for drugs administered through different routes such as systemic drug delivery based on enteral, parenteral, or transdermal.[3, 145] In transdermal application for example, drugs are delivered through penetration of skin barrier stratum corneum (less than 50 nm pore size) into blood circulation by fusion between skin and carrier lipid layers. Carriers with good skin permeation are needed for this purpose.[146] Examples of transdermal delivery applications are treatment of skin cancer, cardiovascular disease, motion sickness, and Parkinson's disease.[147] Vesicles such as niosomes and/or liposomes with added alcohols, or embedded in a hydrogel network were deemed as suitable carrier candidates.[147, 148] Hydrogels are hydrophilic 3-dimensional cross-linked (physically through hydrogen bonding, ionic or hydrophobic interaction; or chemically through covalent bonding) polymeric network that absorbs large quantity of water and is insoluble in aqueous solution. Smart or responsive hydrogels that change properties (undergo reversible volume or sol-gel phase-transition) in response to external environmental stimuli such as pH, temperature, electric field, ionic strength, biomolecules, and light has proved particularly attractive for sensor and diagnostic purposes.[149]

Park and coworkers recently showed that anti-oxidant loaded ceramide liposomes embedded in cellulose hydrogel has greater anti-oxidant skin permeability compared to single system using either liposome or hydrogel.[150] This approach has similarly been applied to deliver skin whitening compound linoleic acid, where high whitening activity was observed.[151] As liposome-in-hydrogel carriers could provide a controlled and sustained drug release,[152] they have been investigated in various forms of drug deliveries. [153, 154] Contrary to liposome-in-hydrogel, a hybrid liposome-hydrogel complex could also be

designed with outer liposomes and inner hydrogels.[155] In this case, the hydrogel interior provides mechanical stability with controlled drug release capability, while the outer liposome layer (with or without incorporated drug) was stealth-modified with conjugated ligands for targeted delivery.[156] Although this principle can be utilized for multiple drug delivery with unique release profiles, preparation of liposome-hydrogel complex with desired properties to achieve controlled release, and its characterisation, is not trivial. Another notable application of vesicular delivery which could revolutionize chemopreventive of breast cancer in high-risk women is through topical administration of breast cancer drugs such as Tamoxifen of which its conventional systemic administration increased the risks of endometrial cancer and thromboembolic events.[157]

Enteral drug delivery route including oral administration involves drug absorption in one of the gastrointestinal/GI tract (stomach, intestine, colon). As such, carriers designed for drug delivery to colon for instance, should be stable against low stomach pH and enzymatic degradation.[158] This has been achieved using pH-sensitive and sustained-release carriers such as functionalised liposomes embedded in pH-sensitive hydrogel matrix or glycolipid-based carriers.[159] In parenteral intravenous drug delivery, although the drug carriers are not be subjected to degradation in GI tract, they need to be injectable and stable against clearance from blood circulation.[160] A particularly attractive carrier for this application is stealth liposomes or vesicles that could improve sustained drug release. However, faster drug release rate could be observed in lamellar vesicles compared to non-lamellar inverted bicontinuous cubic phase due to higher viscosity.[161] Cubosomes, formed by dispersion of surfactant inverted bicontinuous cubic phase in aqueous solution, have thus been explored as potential drug delivery vehicle in oral, topical, transdermal and parenteral administration due to their unique properties.[162] They are less viscous than the bulk cubic phase and is therefore injectable, have high drug loading capacity due to high interfacial areas, could incorporate both hydrophilic and hydrophobic drugs, protect the drugs from degradation in GI tract, and release them in a controlled manner.[163] Interestingly, there are also studies that observed burst release behavior of hydrophobic drugs in cubosomes due to their sub-micron length scales, which could limit its application in prolonged drug release. This could potentially be overcome through modulation of the cubosome properties by changing the compositions or through surface modification.[119]

In addition to the recent advances in liposomes and vesicles-drug delivery through the above-mentioned routes, another novel perhaps one of the most promising applications of liposomes and glycolipid vesicles is to deliver drugs through biomedical implants.[164] The liposomes could be immobilised onto biocompatible and biodegradable implant materials to provide a controlled drug release for treatment of inflamed tissues, infections, or even cancers.[165] As implant materials are inserted close to or at the site of target, anti-cancer drugs delivered through this method are not subjected to problems commonly encountered in intravenous and oral drug delivery such as fast clearance, degradation and possible side effects caused by healthy tissue distribution. This approach has also been proved beneficial for unresectable cancers;[166] and the use of liposomes or vesicles in implanted drug delivery for disease treatment could be a subject of intense research in the near future.

6. Liposomes and vesicles as diagnostic agents

Liposome and glycolipid vesicles, besides being good platform as therapeutic drug carriers as has already been discussed, can also serve as targeted diagnostic agents or a combined imaging and therapy. This popular field has shown much promise in disease diagnostic applications particularly in early cancer detection.[167] Different diagnostic agents transported by liposomes or vesicles could be imaged using tools shown in Table 3 such as ultrasonography (US), magnetic resonance imaging (MR), positron emission tomography (PET), single-photon emission tomography (SPECT), and optical imaging such as visible light and near infra-red (NIR) fluorescence.[168]

Table 3 Various *in vivo* imaging techniques (PET: positron emission tomography, SPECT: single-photon emission tomography, NIR: near infrared, MR, magnetic resonance), their features and limitations

Method	Feature	Limitation
	Nuclear imaging	
PET[169]	Vesicle carries positron- emitting radionuclides (^{11}C, ^{13}N, ^{15}O, ^{18}F). Deep tissue imaging.	High cost, radio-isotope handling.
SPECT[169]	Vesicle carries radionuclides (^{99m}Tc, ^{123}I, ^{111}In). Deep tissue imaging.	Radio-isotope handling, lower sensitivity and spatial resolution than PET.
	Optical imaging	
Visible light (wavelength 400-700 nm)[170]	Vesicle carries bioluminescence probes (photoproteins).	Light penetration through tissue only few mm. Application limited to skin, breast tissue and small animal imaging.
NIR (wavelength 600-950 nm)[171]	Vesicle carries NIR dye (cyanine dyes, phthalocyanines). Tissue penetration mm-cm. Low tissue auto-fluorescence.	High degree of light scattering in tissue.
	Other imaging modes	
MR[172]	Vesicle carries MRI contrast agent (paramagnetic, superparamagnetic molecules). High spatial resolution.	Lower sensitivity than optical and nuclear imaging.
Ultrasound[172]	Vesicle carries ultrasound contrast agent (gas-filled).	Ultrasound waves cannot pass through many body parts such as bone and gas-filled bowel.

For PET and SPECT imaging, radioisotope labels can be encapsulated in or tethered to ligand-conjugated liposome surface. This allows *in vivo* tracing of the liposome circulation time, tumour penetration visualization, biodistribution and clearance kinetics.[173] ^{111}In, ^{99m}Tc, and ^{64}Cu radiotracers labelled to PEGylated liposomes have successfully used to image tumour-bearing rats. Examples of different radionuclides used for SPECT and PET imaging, and liposomal labeling have recently been reviewed in [174]. As radioisotopes could be toxic *in vivo* and are dangerous to handle, PET, MR, and the rapidly growing NIR imaging tools are more widely used. Fluorescence imaging in the far-red/NIR region (600-950nm) was reported to be beneficial for image-guided *in vivo* liposomal drug delivery

because of deep NIR penetration into tissues of up to 10 cm, and low tissue autofluorescence thus high signal-to-noise ratio. This technique can therefore be used to visualise cells directly through tissue or skin.[171] With the recent development of multimodal imaging techniques (MR/optical, MR/CT, SPECT/optical, SPECT/MR) to obtain complementary information and improved disease diagnosis, multi-functional liposomes that can bind (to outer surface of liposome or ligands) and entrap various contrast agents have been designed.[175] A single liposome system for instance, could be functionalized with paramagnetic Gadolinium metal ion (a MRI contrast agent), ^{99m}Tc (a PET contrast agent), and an IRDye (a NIR probe). [175] It is therefore clear that vesicles have broad applications in biomedical and diagnostic research, and could make great contributions towards breakthroughs in science.[121]

7. Conclusions

Whilst it is tempting to cover all the aspects of nano-carriers for controlled deliveries, our review serves to provide valuable insights into utilising self-assembled liquid crystal structures for disease targeting and diagnostic purposes. Molecular self-assembly of amphiphilic molecules such as lipid-and surfactant based materials can be used to build efficient carriers for targeted delivery and release of bioactive molecules in a vast array of applications ranging from pharmaceutical to cosmetics. Of particular interest, naturally derived sugar surfactant compounds have become an important class of nano-carrier in modern drug delivery systems although much work are still required to validate the applicability of these bio-surfactants in various routes of human system-drug administration.

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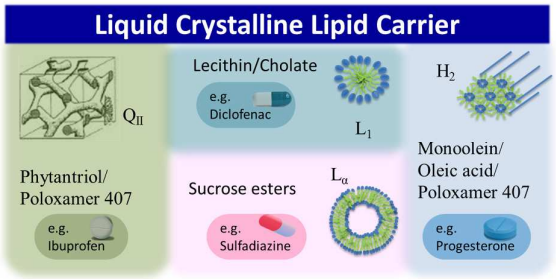
Graphical abstract



Our review highlights lipid liquid crystal nanocarriers, essentially their design considerations and sugar-based materials for specific targeted delivery.