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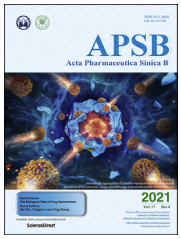

REVIEW

Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles

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Solid crystalline phases;
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Abstract The use of lipid nanocarriers for drug delivery applications is an active research area, and a great interest has particularly been shown in the past two decades. Among different lipid nanocarriers, *ISAsomes* (Internally self-assembled somes or particles), including cubosomes and hexosomes, and solid lipid nanoparticles (SLNs) have unique structural features, making them attractive as nanocarriers for drug delivery. In this contribution, we focus exclusively on recent advances in formation and characterization of *ISAsomes*, mainly cubosomes and hexosomes, and their use as versatile nanocarriers for different drug delivery applications. Additionally, the advantages of SLNs and their application in oral and pulmonary drug delivery are discussed with focus on the biological fates of these lipid nanocarriers *in vivo*. Despite the demonstrated advantages in *in vitro* and *in vivo* evaluations including preclinical studies, further investigations on improved understanding of the interactions of these nanoparticles with biological fluids and tissues of the target sites is necessary for efficient designing of drug nanocarriers and exploring potential clinical applications.

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1. Introduction

Explosive growth of research on the use of lipid nanoparticles in the development of nanocarriers for drug delivery and biomedical imaging purposes has been witnessed over the last 30 years[1−6]. For therapeutic and diagnostic applications, the attractiveness of lipid nanoparticles relies among others on the capability of loading therapeutic and diagnostic agents, protecting them from degradation, enhancing absorption and improving intracellular penetration, minimizing systemic toxicity, modifying pharmacokinetics, and overcoming systemic and tumor barriers[1,4,7]. They are particularly attractive for loading poorly water-soluble drugs that typically display limited bioavailability, poor pharmacokinetics, and adverse side effects[2,4]. Surface engineering can also be employed through different surface manipulation strategies on lipid nanoparticles for active drug targeting[1,2,4,5,7].

Beside liposomes, many efforts have been devoted in the utilization of emulsions, micellar solutions including microemulsions, solid lipid nanoparticles (SLNs), and non-lamellar liquid crystalline nanoparticles (mainly cubosomes and hexosomes) in the development of nanocarriers for drug delivery applications[1,2,4,5,7−14]. Among these nanoparticles, we exclusively focus in the present contribution on major challenges and recent advances in the use of non-lamellar liquid nanoparticles (mainly cubosomes and hexosomes) and SLNs in the development of nanomedicines. We discuss also future opportunities and potential fate of these nano-self-assemblies *in vivo*.

Non-lamellar liquid crystalline nanoparticles are nano-self-assemblies, sharing common features with SLNs and typically require the use of an efficient stabilizer for their colloidal stabilization in excess water[6,8,9,11,15]. In SLNs, the internal architectures are solid crystalline phases[9,11,12,16]; whereas cubosomes and hexosomes envelope internally non-lamellar liquid crystalline phases (inverse bicontinuous (Q_2) and discontinuous hexagonal (H_2) phases, respectively)[6,8,15,17]. Fig. 1 illustrates the features of non-lamellar liquid crystalline nanoparticles and SLNs.

Lowering the melting point of the solid matrix in SLNs can be achieved by modifying the internal architectures of SLNs through partial replacement of the used single solid lipid (or solid lipid combination) with liquid lipids (typically triglycerides) to produce nanostructured lipid carriers, which are still solid at room and body temperatures[9,16,18]. However, such modifications in lipid compositions and the involved transformation of SLNs to nanostructured lipid carriers are generally associated with alterations in the structural features of the solid crystalline phases. For the production of SLNs, saturated fatty acids, saturated monoglycerides, and saturated triglycerides are typically used as solid lipid cores; whereas Pluronics and Tween 80 are among the most used stabilizers[9,11,12,16,19]. However, cubosomes and hexosomes are generally produced from single amphiphiles (or amphiphile combinations) with high propensities to form inverse non-lamellar liquid crystalline phases at room and body temperatures, and typically stabilized with Pluronic F127[6,8,15,17,20−24]. In their preparations, the most used amphiphiles include unsaturated monoglycerides, phytantriol, and their combinations with oils such as vitamin E and oleic acid[6,8,15,17,24−31]. Taking into account the aforementioned common features, and high sensitivity of internal phases (solid crystalline phases and inverse non-lamellar liquid crystalline phases of SLNs and *ISAsomes*, respectively) to alterations in lipid types and compositions, and solubilization of drugs, this contribution focuses on recent advances in drug delivery applications of these families of lipid nanoparticles.

Regarding the size characteristics, SLNs can be produced with sizes in the range of 40−1000 nm[9,12,13,16]. Similar to non-lamellar liquid crystalline nanoparticles (having typically sizes in the range of 100−200 nm)[6,8,22,27,30,32−34], the mean nanoparticle sizes and size distributions in SLN preparations are mainly affected by the used lipid type and composition, the stabilizer type and concentration, and the employed emulsification method[11,16,18,35]. In addition to the typical low- and high-energy emulsification methods (including ultrasonication and high-pressure homogenization) for SLN, cubosome and hexosome preparations[6,9,11,12,14−16,18,23,27,32,36], other emulsification methods including modified high-pressure homogenization methods have been introduced for SLN production[11,16,35]. There is also a growing interesting in utilization microfluidics for controlling the size and size distributions of these solid and non-lamellar liquid crystalline nanoparticles[37−41].

2. Cubosomes, hexosomes, and related nano-self-assemblies

The family of structurally tunable nanoparticles enveloping internally inverse non-lamellar liquid crystalline phases or micellar phases, known in literature as *ISAsomes* (Internally self-assembled somes), include cubosomes, hexosomes, micellar cubosomes, and emulsified microemulsions (EMEs)[6,8,15,17,26,32,42−48]. These nano-self-assemblies have interior architectures of inverse bicontinuous cubic (Q_2) phases, inverse discontinuous hexagonal (H_2) and cubic *Fd3m* phases, and inverse microemulsions (water-in-oil microemulsions, L_2 phases), respectively[6,8,15,17,45]. These colloidal nano-objects are also of biological relevance and their generation during digestion of triglyceride-containing food products including milk and mayonnaise, and model food emulsions has been discussed in different previous reports[17,49−53]. It was proposed that they act as nanostructured carriers for facilitating the delivery of poorly water-soluble nutrients including vitamins[53].

2.1. Formation and characterization of ISAsomes

Alongside liposomes, the most investigated lipid nanoparticles for drug delivery and bio-imaging applications, there has been an increased interest in exploring *ISAsomes* (mainly cubosomes and hexosomes) as attractive versatile nanoplatforms for biomedical and pharmaceutical applications[5,6,8]. These nanodispersions are typically formed in the presence of an efficient stabilizer by applying high-energy emulsification methods that include ultrasonication, microfluidization, and high-pressure homogenization[6,15,17,20,54]. In the 1990s, Larsson and coworkers[23,24] reported the first studies on formation and characterization of Pluronic F127-stabilized nanodispersions (cubosomes and hexosomes) that were produced using a high-energy emulsification method. It is also possible at certain lipid compositions to produce these colloidal nanoobjects by applying a low-energy emulsification method based on vortexing the single lipid (or the lipid combination) in excess water[32,36,55]. Following a similar approach, known in literature as the bottom-up approach, it is possible to produce these nanoparticles by adding a suitable hydrotrope, prior to the application of the low-energy emulsification method[56]. There is also an interest in the continuous production of these nano-self-assemblies by using microfluidics, and also coupling specially designed microfluidics with synchrotron SAXS for real-time determination of the involved dynamic

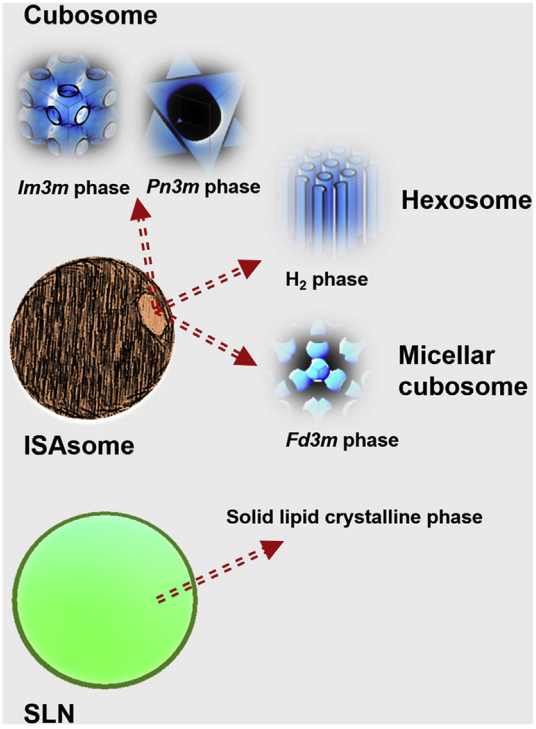

Figure 1 Schematic illustration of representative nano-lamellar nano-self-assemblies with unique structural features and solid lipid nanoparticles (SLNs). These nanoparticles are attractive for use as nanocarriers for drug delivery and bio-imaging applications.

structural features during their evolvement[38–40]. In addition to Pluronic F127, various stabilizing agents including other Pluronics (such as F128), PEGylated lipids, β-casein, and citrem have been introduced for the stabilization of cubosomes, hexosomes and other *ISAsomes*[6,15,23,32,47,54,57–60]. Among these agents, citrem, a food-grade emulsifier, was found to be an attractive agent in the development of immune safe *ISAsomes* owing to its hemocompatiblity (poor activation of the complement system and lack of hemolytic effects)[32,59]. Taking into account the reported major limitations (including poor hemocompatibility and cytotoxicity) of most investigated Pluronic F127-stabilized cubosomes and hexosomes[6,33,59,61,62], it is worth using citrem as an attractive alternative stabilizer, particularly for nanodispersions intended for intravenous drug delivery applications[32,59]. In a recent report on utilizing mPEG-lipid conjugates as stabilizing agents of *ISAsomes*, it was found that their lipid moieties may have modulatory effects on nanoparticle-mediated complement activation[47]. Among different mPEG-lipid conjugates, it was reported that TPGS-mPEG$_{2000}$ is the most attractive lipopolymer for effectively overcoming complement activation[47]. In another recent study, Murgia and co-workers[63] introduced polyphosphoester analog of Pluronic F127 as an alternative stabilizer for production of cubosomes with reduced cytotoxicity. Stabilizer-free cubosomes at certain compositions were also recently produced and characterized[64]. For further information on the most investigated lipids and stabilizers in *ISAsome* preparations, the readers are directed to the following reviews[6,8,15,17,20,65].

A toolbox of different state-of-art biophysical techniques are typically used to gain important information on the structural and morphological features, and size characteristics of *ISAsomes*[6,8,15,17,20,33]. Among these techniques, we mention small-angle X-ray (SAXS) and neutron (SANS) scattering techniques, cryogenic transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS), and nanoparticle tracking analysis (NTA) as routine and most used techniques in this research area[6,8,17,20,33,66]. SAXS and SANS are well-established powerful tools for structural characterization of soft matters including *ISAsomes* and their corresponding non-dispersed (bulk) inverse liquid crystalline (or micellar) phases[6,15,17,20,65,67,68]. In addition to SAXS and SANS static investigations[6,15,20,34,69–71] focusing among others on the effects of temperature, pH, aqueous medium composition, lipid composition and type, and drug type and concentration, there is an interest in investigations under non-equilibrium conditions. The latter investigations focus on gaining insight into the involved dynamic structural alterations during the *in situ* production of such nano-self-assemblies[38,40] or on their exposure to biologically relevant fluids, cell culture models, or buffers containing ions[49,72–75]. For instance, synchrotron SAXS was recently coupled with a suitable microfluidic platform for monitoring in real time dynamic structural features during the continuous production of these nano-self-assemblies[38]. As a complementary technique, cryo-TEM is typically combined with SAXS or SANS and provides important information on the morphological features of *ISAsomes*[6,20,47,66,76]. It is considered a direct method and its importance relies not only on morphological characterization of *ISAsomes* and gaining insight into their self-assembled interiors through Fourier transformation analysis (FFT), but also on the characterization of the surface properties of these nanoparticles and shedding light on possible coexistence of other nanoobjects including micelles and vesicular structures[6,34,47,66,76,77]. The surface characteristics are related to the presence of vesicles or sponge phases adhering the outer surfaces of *ISAsomes*, which are known as surface phases[20,32,34,47,78,79]. For gaining information on nanoparticle sizes and size distributions, DLS and NTA are typically employed and combined with SAXS (or SANS) and cryo-TEM[6,20]. Representative examples on the use of NTA, SAXS, and cryo-TEM for the characterization of size characteristics, and structural and morphological features of *ISAsomes* are shown in Figs. 2 and 3.

2.2. *ISAsomes as versatile nanocarriers for drug delivery*

The last decade has witnessed a growing interest in the utilization of *ISAsomes*, mainly cubosomes and hexosomes, as nanocarriers for loading various drugs, imaging probes, and antimicrobial peptides[5,6,8,34,40,45,69,70,80–88]. In particular, a great attention has been directed towards their use for enhancing the solubilization of poorly water-soluble drugs, including curcumin, thymoquinone, and cinnarizine[6,70,78,81,89,90]. However, most investigations focused on the encapsulation efficiency and the impact of loaded drug type and concentration, lipid composition, and stabilizer type and concentration on the structural and morphological features, and size characteristics of these nano-self-assemblies by typically

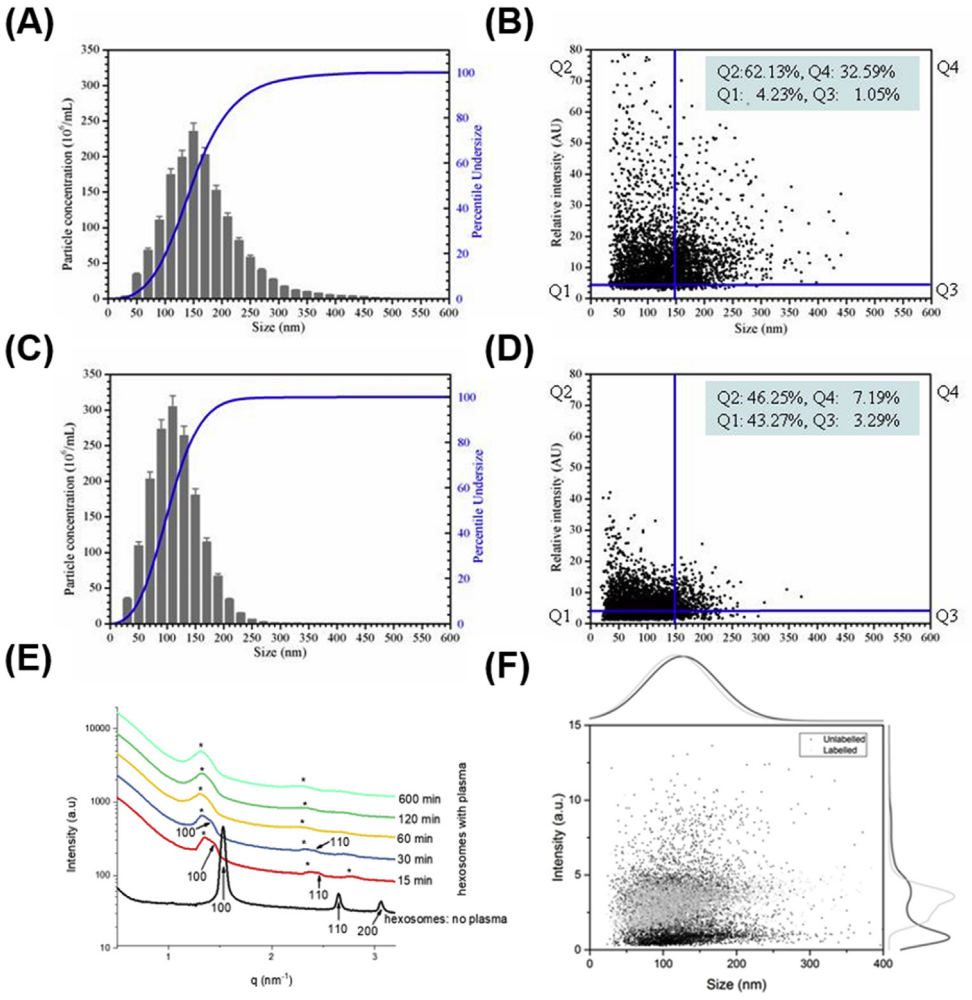

Figure 2 Size and structural characterization of *ISAsomes*. NTA results showing size distribution profiles (panels A and C), and relative light scattering intensities (panels B and D), before (panels A and B) and after (panels C and D) incubation of F127-stabilized PHYT nanodispersion with human plasma. In the presence of plasma, comparison of size distribution profiles indicated that plasma-mediated loss of some relatively larger nanoparticles (≥150 nm) with concomitant decrease in intensity. Characterization of F127-stabilized PHYT/oleic acid (OA) hexosomes by synchrotron SAXS (E) and NTA (F). In panel E, black colored SAXS pattern indicating the formation of unlabelled hexosomes at 37 °C, and effect of swelling at different time points on incubation of the nanodispersion with rat plasma. (F) Relative light scattering intensity for unlabelled (black) and (^{99m}Tc)-labelled hexosomes (grey). Panels A−D were taken with permission from Ref. 33; whereas panels E and F were taken with permission from Ref. 30.

combining SAXS with cryo-TEM and DLS (or NTA) as mentioned above[6,8,34,45,69,70,78]. The number of studies on their drug release properties, cellular responses, and other *in vitro* evaluations is still relatively limited. Their cellular uptake efficiency (including cellular uptake mechanisms), and cytotoxicity are only evaluated on relatively limited number of cell lines, mainly cancer cell lines[5,6,32,46,61,62,91−95]. It is still worth mentioning that there is an increasing interest in these evaluations by different research groups in the last few years. However, further investigations should be conducted to gain insight into the effects of lipid composition and type, and stabilizer type and concentration on the cellular uptake mechanisms of these nano-self-assemblies. The *in vivo* fate of these nanoparticles, particularly those developed for parenteral applications, is still scarcely investigated, and the influence of the physicochemical properties (including nanoparticle size characteristics, structures, and surface properties) and administration route on their biodistribution and cellular uptake is still largely unexplored. In this sub-section, we present different examples on *in vivo* evaluations of cubosomes and hexosomes and highlight the most important aspects. Selected examples are also presented in Fig. 4. Here, we focus on oral, intravenous, and subcutaneous drug delivery applications. For further information on other applications of cubosomes, hexosomes, and related nanoparticles, including topical, trans- and intra-nasal, ophthalmic, and skin drug delivery, and their uses in the development of theranostic nanocarriers, the readers are directed to relevant reports and recent review articles[5,6,8,17,45,87,96−99]. In addition to *ISAsomes*, there is an interest in the utilization of *in situ* forming drug delivery systems based on inverse lyotropic non-lamellar liquid crystalline phases for drug delivery applications[6,31,100−105]. They are attractive in the design of parenteral formulations with tunable nanostructures and sustained release properties[98,101−105].

2.2.1. *Oral drug delivery*

In the development of nanoparticles intended for oral drug delivery applications, previous studies reported through *in vitro* and *in vivo* evaluations on an improved bioavailability and sustaining

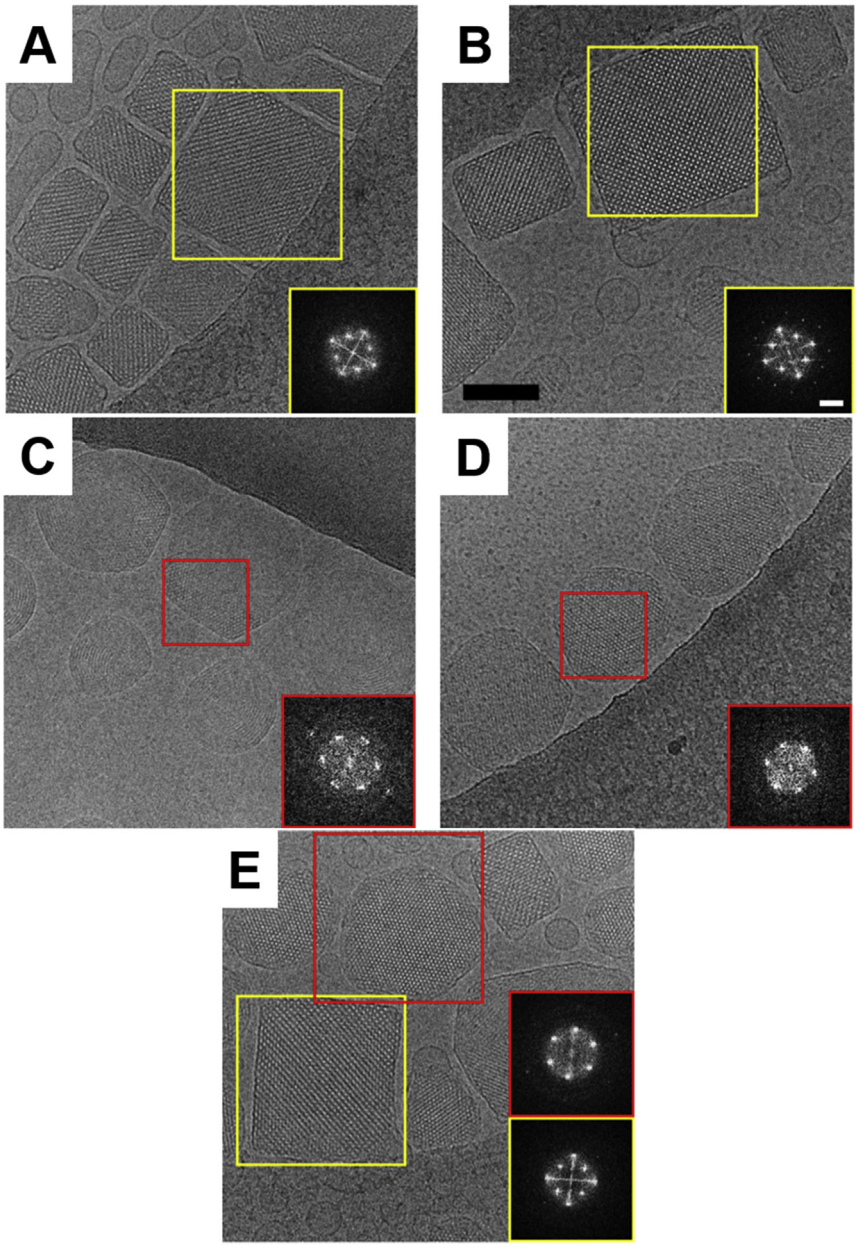

Figure 3 Morphological characterization of *ISAsomes*. Cryo-TEM images of selected F127-stabilized nanodispersions after vitrification at 37 °C. (A) unlabeled PEGylated PHYT nanodispersion; (B) labelled PEGylated PHYT nanodispersion with ^{99m}Tc-HMPAO (technetium-99 m labelling by using the chelating agent hexamethylpropyleneamine oxime, HMPAO); (C) unlabeled PHYT/OA hexosomes; (D) unlabeled PHYT nanodispersion; (E) unlabeled PHYT/1, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) nanoparticles. Red insets reveal the fast Fourier transformation (FFT) analysis of the observed hexosomes displaying internal H_2 phase, whereas yellow insets display FFT analysis of cubosomes with internal inverse bicontinuous cubic *Pn3m* phase. Scale bar: 100 nm. This figure was adapted with permission from Ref. 118.

release of various drugs [including 20(*S*)-protvopanaxadiol, doxorubicin, and cinnarizine] loaded to cubosomes or hexosomes[89,106–108]. The evaluated nanoparticles were based on either phytantriol (PHYT) or monoolein (MO) and stabilized with Pluronic F127. For example, it was reported after an oral administration of doxorubicin-loaded PHYT cubosomes to rats on an improved bioavailability, an improved antitumor efficacy, and a lower level of cardiotoxicity as compared to the FDA-approved formulation Adriamycin®, which was intravenously administered[109]. This improved oral doxorubicin delivery was attributed to a longer circulation half-life and an improved tumor accumulation of nanoparticles *via* an enhanced permeation and retention (EPR) effect[109]. In another example, Yang et al.[110] reported on an improved oral delivery of amphotericin B loaded to MO cubosomes for anti-fungal infection treatment. As compared to the clinical formulation Fungizone®, which was intravenously administered, a more significant efficacy was reported for the orally administered cubosomal formulation. In general, an improved oral bioavailability was not only reported for cubosomes and hexosomes, but also for other orally administered lipid nanoparticles[45]. It is most likely attributed to the ability of the lipid cores in these lipid nanoparticulate formulations to stimulate

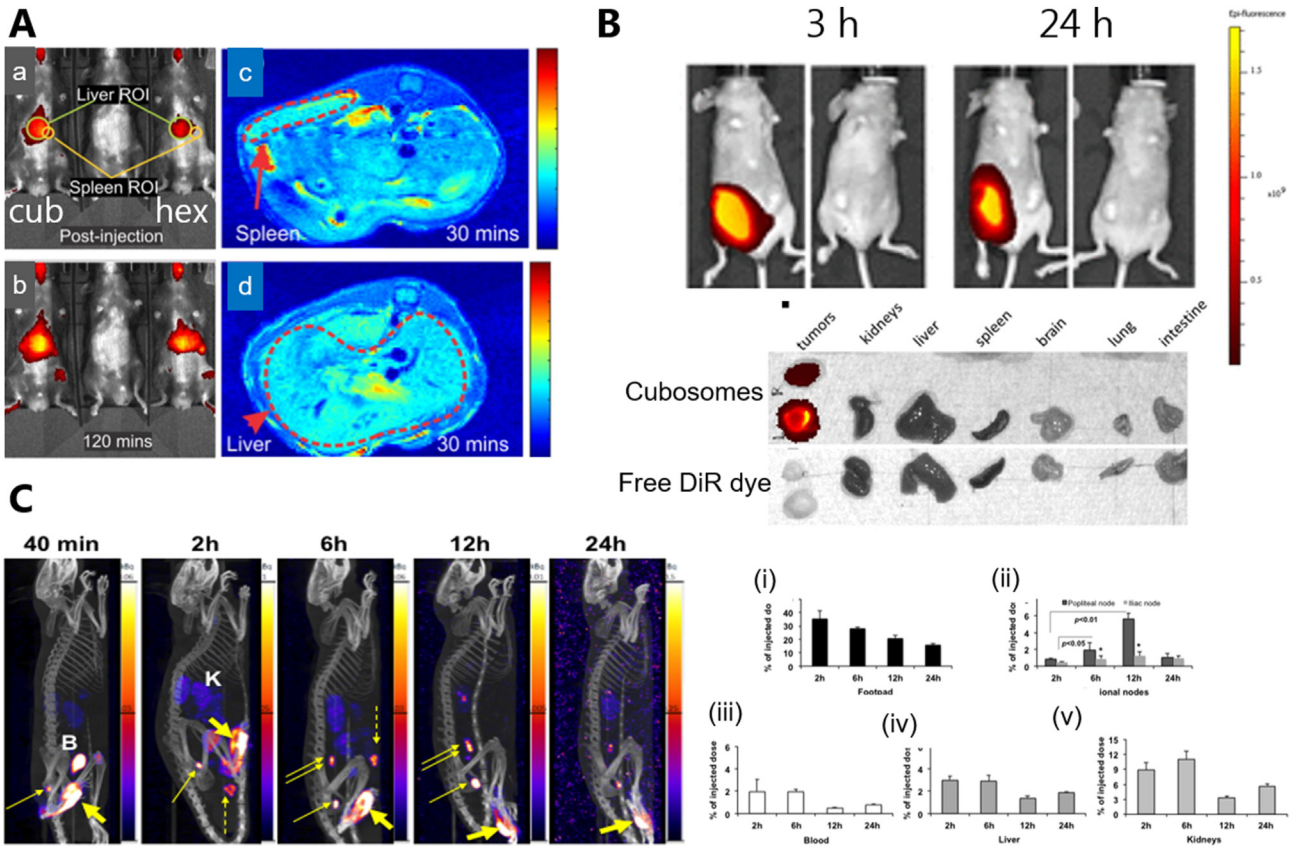

Figure 4 (A) NIRF imaging of whole body of mice after tail vein injections of cubosomes (cub) and hexosomes (hex): a) immediately after injection, and b) at 120 min of post injection. In c and d, enhanced *in vivo* MRI images (marked by dotted lines) of spleen and liver at 30 min post-injection of NIRF-MRI cubosomes are shown. Adapted with permission from Ref. 111. (B) In the left: whole body imaging of A431 tumor-bearing mice at 3 and 24 h of i.p. post-administration of paclitaxel-containing cubosomes. In the right: *ex vivo* imaging of organs at 24 h of post-injection. Adapted with permission from Ref. 115. (C) Whole body SPECT/CT imaging and biodistribution of ^{99m}Tc-labeled-hexosomes on footpad s.c. injection. Thick yellow arrows indicate accumulated formulation at the footpad site. Single yellow solid arrow = popliteal node; double yellow solid arrow = iliac node; dashed arrow = inguinal node; K = kidney; B = bladder. Biodistribution: radiotracer contents in the whole footpad, regional lymph nodes, blood pool, liver, and kidneys at different post-injection times, as determined by γ-counting, are shown in panels (i)–(v). Adapted with permission from Ref. 30.

secretion of bile and pancreatic enzymes within the small intestine that facilitates digestion and leads to the generation of mixed micelles, which enhance the transport of loaded drugs and improve their absorption rates[45,53].

Despite the attractiveness of cubosomes, hexosomes, and related nanoparticles as oral drug nanocarriers owing to the reported improved bioavailability and enhanced efficacy, the involved mechanisms after oral administration and the roles of the structural features and physicochemical properties, including nanoparticle size and charge, and surface characteristics are still largely unexplored[45].

2.2.2. *Intravenous drug delivery*

Among the few published studies, we mention the recent work on the combination of magnetic resonance (MR) and near infrared fluorescent (NIRF) imaging modalities for development of F127-stabilized cubosomes and hexosomes as agents with dual MR-NIRF imaging properties[111]. Through NIRF imaging[111], the *in vivo* biodistribution of these nanoparticles was investigated after an intravenous (i.v.) administration to mice. It was found that the administered nanoparticles accumulated up to 20 h of post-administration in the liver and spleen of mice (Fig. 4A). However, the accumulation level seems to be dependent on the lipid composition and/or structural features as hexosomes showed a greater level of accumulation in the spleen than the liver as compared to cubosomes[111]. A possible difference in the stabilizer F127 surface coverage of hexosomes as compared to cubosomes may also play role in the observed difference in their accumulation behavior. Such difference may lead to a more preferential recognition of cubosomes by Kupffer cells in the liver[111]. The preferred accumulation of cubosomes in liver after i.v. administration of MO cubosomes to mice was also confirmed in the first report on NIRF *in vivo* imaging of i.v. administered cubosomes[112]. In addition to the investigated biodistribution of cubosomes and hexosomes, *in vivo* MR imaging indicated an enhanced contrast in the liver and spleen[111]. An enhancement of MR contrast for *in vivo* imaging was also reported for nitroxide-loaded MO hexosomes[113]. In another study, Jain et al.[114] reported on radiolabeling of PEGylated non-lamellar liquid crystalline nanoparticles loaded with paclitaxel [^{99m}Tc-(Technetium radionuclide)-labeled nanoparticulate formulation], and evaluating their biodistribution after i.v. administration to Ehrlich Ascites

tumor (EAT)-bearing mice. They found that PEGylation of these nanoparticles is not only associated with an enhanced safety, but also contributes to an improved circulation time and enhanced tumor accumulation by EPR as compared to corresponding non-PEGylated cubosomes and plain paclitaxel[114]. The observed tumor growth inhibition with the non-PEGylated nanoparticles was attributed to their internalization into the tumors through EPR and other non-specific effects. PEGylation was associated with a higher level of tumor growth inhibition due most likely to EPR owing to prolonged circulation, and sustained release of paclitaxel from the lipid nanocarriers. In a recent report[115], the *in vivo* biodistribution of MO cubosomes loaded with paclitaxel was also investigated. However, the investigations were done after intraperitoneal (i.p.) administration of the nanoparticles to mice. It was reported on an enhanced tumor accumulation and a reduction of tumor average size following i.p. administration of paclitaxel-containing cubosomes as compared to corresponding control (paclitaxel-free) cubosomes (Fig. 4B). In addition to paclitaxel-loaded nanocarriers, F127-stabilized MO cubosomes were suggested as suitable candidates for loading etoposide, which is a topoisomerase II inhibitor displaying anti-proliferative activity[116]. *In vivo* investigations on i.v. administered etoposide-containing folate-modified and unmodified cubosomes to mice bearing human breast carcinoma MCF-7 indicated that nanoparticle modification with folate was associated with an improved anti-proliferative activity as compared with unmodified cubosomes and free etoposide. For the unmodified nanoparticles, the tumor accumulation was attributed to EPR; whereas the folate-modified nanoparticles had an improved tumor-targeting ability, which is attributed most likely to interactions of folate with folate receptor overexpressed on the surface of MCF-7 breast cancer cells[116].

Similar to other nanoparticles[117], the exhibited high tendency of unmodified cubosomes and hexosomes to accumulate in liver and spleen (organs rich with reticuloendothelial cells), particularly after i.v. administration, is most likely attributed to the opsonization process.

2.2.3. *Subcutaneous drug delivery*

A highly efficient surface chelation method with good radiolabeling (84%) and high radiochemical purity (>90%) was employed to radiolabel Pluronic F127-stabilized hexosomes with technetium-99 m (^{99m}Tc) in the presence of 12-diamino-3,6,9-triazododecane (SpmTrien) as a chelating agent[118]. In two reports, the synthesized ^{99m}Tc-SpmTrien-hexosomes were evaluated for *in vivo* imaging after subcutaneous (s.c.) administration to right flanks of healthy mice[118] and footpads of healthy rats[30] by using single photon emission computed tomography in combination with computed tomography (SPECT/CT). It was reported that the radiolabeling procedure did not affect the mean nanoparticle sizes, and structural and morphological features of hexosomes. However, NTA suggested a slight change in nanoparticle size distribution, which is most likely attributed to the loss of some coexisting vesicles during the labeling procedure. In the first set of investigations, the *in vivo* biodistribution within 24 h of post-administration of ^{99m}Tc-SpmTrien-hexosomes to mice indicated the high stability of these nanoparticles, the formation of a deposit within the subcutaneous adipose tissue and the neglected biodistribution in other organs and tissues[118]. In the follow-up study[30], the injection of ^{99m}Tc-SpmTrien-hexosomes into the footpads of rats led to their rapid drainage into the lymphatic microvessels and biodistribution not only to the sentinel (popliteal) lymph node, but also to wider lymph nodes (inguinal, iliac) situated along the pathway of the lymph drainage (Fig. 4C). Thus, as compared to conventional drug nanocarriers, hexosomes may provide promising and simple-by-design nano-self-assemblies for the development of lymphotropic and multifunctional nanocarriers without surface nano-engineering with targeting ligands[30]. It was suggested that Pluronic F127, covering the outer surfaces of hexosomes, may play a modulatory role in the detected rapid drainage from the footpad interstitium and the simultaneous recognition by the lymph node macrophages. It was proposed that the surface projected ethylene oxide (PEO) blocks of F127 may display a "mushroom-like" configuration in hexosomes, leading to minimization of interactions within the footpad interstitium, without interfering with lymph node macrophage recognition. Further information, regarding the modulatory role of PEO configuration, can be found in a previous report on lymphatic performance of F127-coated nanospheres[119].

In the development of nanoparticulate formulation for liver targeted drug delivery, Pluronic F127 MO cubosomes loaded with 5-fluorouracil was evaluated after s.c. injection into rats[120]. After 3 h of administration, it was reported on an enhanced accumulation of 5-fluorouracil (about 5-fold increase) in the liver as compared to an aqueous solution of this drug[120]. However, the increase in drug concentration was associated with hepatocellular damage. A high permeability of cubosomes to the epithelial membrane may play role in the observed liver uptake[120]. In recent studies, it was also reported on evaluation of cubosomes after s.c. administration and their attractiveness in the design of nanocarriers for vaccine delivery applications[121,122].

3. Solid lipid particles as nanocarriers for drug delivery

SLNs have shown great application potentials in encapsulating both lipophilic and hydrophilic drug molecules, controlling drug release, and targeting drug delivery to specific cells and tissues[14,123–126]. The major excipients of the SLNs are solid lipids at room temperature, containing typically long-chain saturated fatty acids as the basic building blocks[125,127]. The slow degradation rate of saturated lipids leads to sustained release of drug molecules encapsulated in SLNs. Clearly, the composition of lipid excipients strongly affects the biological fate of embedded drug molecules in the solid crystalline matrix as well as SLNs. Additionally, the efficiency of these drug nanocarriers in drug delivery also depends on the delivery route. Interactions among SLNs and components in different biological fluids may lead to a degradation of these nanoparticles or a formation of corona on their surfaces. Such effects may be associated with alterations in the physicochemical properties and functionalities of these nanocarriers[125,128–132]. Therefore, the fate of the nanocarriers is not only depending on nanoparticle composition and structure, but also on the *in vivo* environment. In this review paper, we summarize recent applications of SLNs with focus particularly on oral and pulmonary drug delivery applications. We aim at providing a better understanding of the biological fate of these solid lipid nanocarriers.

3.1. *Solid lipid nanocarriers in oral drug delivery*

Lipid nanoparticulate formulations are often used for oral delivery of poorly water-soluble drugs. This research area takes into account that major lipid excipients may stimulate the release of bile salts and digestive enzymes in the gastrointestinal tract and the

generated lipid digestion products may play an important role by assisting in solubilizing drug molecules and improving their absorption[133,134]. One of the advantages of solid lipid nanocarriers over other kind of lipid-based nanoparticulate formulations is a better control of drug release from the lipid nanoparticles and an improved limitation of the maximum plasma concentration of drugs (C_{max}), and thereby an improved reduction in potential toxicity of drug substances. Delayed time to reach maximum drug concentration (T_{max}) has been reported in several pharmacokinetic studies[124,135,136], for example the T_{max} for cyclosporine after oral administration of drug-loaded SLNs and microemulsion was 4.0 and 1.7 h, respectively[124]. Pharmacodynamic study has also shown a prolonged therapeutic effect after an oral administration of drug-loaded SLNs[137]. Even though various *in vivo* studies have shown that SLNs can improve drug absorption and bioavailability[125,138–141], understanding the involved mechanisms and the biological fates of these nanoparticles is still limited.

In vivo studies have shown that the solid lipid nanoparticle size affects the rate of drug release and absorption: smaller solid lipid nanocarriers generally lead to faster drug absorption and higher bioavailability as compared to relatively larger nanoparticles. For example, the T_{max} of the lipophilic drug torcetrapib was 1 and 2 h after an oral administration of SLNs with nano- and micro-particle sizes of about 150 nm and 8 μm, respectively[142,143]. In these studies, the solid lipid nanocarriers led to a better bioavailability as compared to solid lipid microparticles[142,143]. *In vitro* lipolysis studies also showed that the recovery of drug substances in the aqueous phase was significantly higher for SLNs than corresponding solid lipid microparticles[143]. This is attributed to a greater specific surface area of SLNs led to a larger extent of lipid digestion and drug release. A good *in vitro* and *in vivo* correlation was observed for the effect of SLN size on drug release and bioavailability of fenofibrate (Fig. 5)[143]. Additionally, drug release from lipid particles is affected by lipid excipient[126,144]. It was reported that a slow digestion of relatively long-chain saturated lipids leads to a reduction in the drug release rate from lipid particles (Fig. 6)[126]. Both the *in vivo* and *in vitro* studies indicate that degradation of SLNs in the gastrointestinal tract simultaneously occurs with drug release from these solid lipid nanocarriers.

The principle of lymphatic transport of dietary lipids is valued in pharmaceutical sciences. In this respect, lipid-based formulations can be used for assisting the lymphatic transport of lipophilic drugs[133,134]. Oral drug delivery *via* the intestinal lymphatic system circumvents the hepatic first pass metabolism and transports the loaded drug molecules directly into the systemic circulation. Selection of lipid excipients is one of the key factors to resemble chylomicrons in the enterocytes and stimulate lymphatic transport (Fig. 7)[134]. Both direct analysis of lymph samples collected from cannulated lymph duct in animals and following a chylomicron flow blocking approach have been used in studies of lymphatic transport of drugs and lipids[145–149]. Different particulate drug carriers have been investigated for their potential in delivery of drugs *via* the intestinal lymphatic system[150,151]. Among these studies, lymphatic transport of efavirenz-loaded SLNs after an oral gavage was evaluated using both chylomicron flow blocking approach and lymph duct cannulated rat model[152]. The investigated SLNs with mean nanoparticle sizes of 170 nm were prepared using long-chain glyceryl dibehenate as a major lipid excipient. Lymphatic uptake of efavirenz was detected in the collected lymph samples, accompanied by a lower drug concentration in the collected plasma samples. This study of Joshi and co-workers indicates that a significant amount of the drug substances in SLNs containing long-chain fatty acids was transported *via* the lymphatic system[152]. However, it was not possible to differentiate and understand whether the nanoparticles were absorbed and transported as intact drug-loaded nanoparticles or an intermediate stage of degradation of lipid nanocarriers in the gastrointestinal tract was involved before drug absorption.

3.2. *Evaluation of solid lipid nanocarriers using imaging and radiotracer techniques*

Imaging and radiotracer techniques have been applied for evaluating solid lipid drug nanocarriers in various studies: from cellular uptake to biodistribution of drug-loaded SLNs *in vivo* after an oral administration. For instance, the biological fate of spironolactone-loaded SLNs in rats after an oral administration was investigated using a radiolabeling method[153]. In this study, the lipid nanoparticles, prepared using a mixture of glyceryl palmitostearate and medium-chain lipids, were labelled by mixing with ^{99m}Tc aqueous solution. After an oral administration of the labelled-SLN suspension, organ and blood associated radioactivity was quantified using a gamma counter. The radioactivity was mainly detected in the small intestine, which was attributed to the retention of solid lipid nanocarriers in the intestinal mucosa[153]. Radiolabelled fatty acid (^{131}I-17-iodoheptadecanoic acid) has also been used as a tracer for monitoring the fate of SLNs after a duodenal administration[154]. The evaluated SLNs were prepared using an emulsification method that includes mixing a warm oil-in-water (O/W) emulsion containing stearic acid with an organic solution of labelled fatty acids. After duodenal administration of SLN suspension in rats, lymph and blood samples were collected. Both lymph and plasma samples showed radioactivity, which was attributed among others to a transport of SLNs into the lymph after duodenal administration to rats[154]. However, degradation of SLNs in the gastrointestinal tract may lead to the release of radiolabeled fatty acids, which could be absorbed either *via* the portal vein to the systemic circulation or *via* the re-synthesis of triglycerides and formation of chylomicrons in the enterocytes that may be transported *via* the lymphatic system.

Fluorescent dye coumarin-6[155], fluorescein isothiocyanate (FITC)[156], and Nile red[157] have been used as probes and added in the lipid phase during the nanoparticle preparation process to track the absorption and metabolism of SLNs. Caco-2 cells are often used as an intestinal model for evaluating drug transport and absorption. Cellular uptake of fluorescently labeled SLNs showed stronger fluorescence signals inside Caco-2 cells when their compositions include medium-chain lipids[155,156]. However, some of the fluorescent probes, such as FITC, can still emit fluorescence after leakage, therefore it can be difficult to use such fluorescence signals as indicators of intact nanoparticles[158]. The increased fluorescence signals inside Caco-2 cells could be caused by a leakage of fluorescent probes from SLNs, especially in the case of nanoparticles containing medium-chain lipids. Recently Hu et al.[159] investigated the fate of SLNs in the gastrointestinal tract by using a combination of water-quenching near-infrared (NIR) fluorescent probes and live imaging techniques. The tested SLNs were prepared by a hot homogenization method, and glyceryl palmitostearate was used as a lipid phase and Tween 80 as an emulsifier. The lipophilic probes were dissolved in an organic solvent and mixed with melted lipids before formation of SLNs. Apart from coumarin 6, two water-quenching NIR fluorescent

probes P2 and P4 were used to label the nanoparticles. Real-time positioning of solid lipid nanocarriers was tracked by using a live imaging system after gastric gavage of the SLN suspension to mice. In this method, the detection was based on a signal switching upon degradation of the lipid matrix and simultaneous release of the probes. The mean solid lipid nanoparticle size was about 75 nm, and the produced SLNs were digested quickly in the intestine and were not able to across the intestinal epithelia[159]. These results are in good agreement with other studies on oral delivery of SLNs, where drug release from the nanoparticles is generally associated with a degradation of these lipid nanocarriers[142,143].

The studies on oral delivery of SLNs suggest that the degradation of nanoparticles occurs in the gastrointestinal tract, and the slow degradation of saturated lipids leads to sustained release of encapsulated drug molecules from these solid lipid nanocarriers. Similar to other lipid-based formulations, the amphiphilic lipid digestion products may assist in solubilizing drug molecules and improve drug absorption. The digested lipids from solid lipid nanocarriers are most likely to be absorbed in a similar way as dietary lipids, *i.e.*, fatty acids could either be bounded to albumin and transported *via* the portal vein to the liver or be re-synthesized to triglycerides in the enterocytes and packed together with lipophilic drug molecules in chylomicrons in the presence of apolipoproteins for enhancing lymphatic transport.

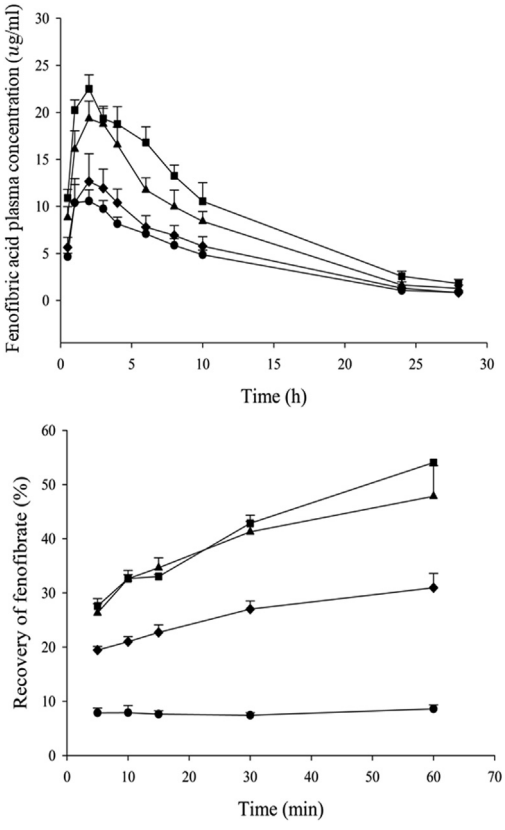

Figure 5 (A) Plasma concentration−time profile following oral administration of 100 nm LMPs (■), 400 nm LMPs (▲), microparticles (◆) and control (●) in male Wistar rats (fenofibrate dosed at 12.5 mg/animal, $n = 6$, mean ± SEM). (B) Recovery of fenofibrate (%) in the aqueous phase (mean ± SEM) during *in vitro* lipolysis of 100 nm SLN (■), 400 nm SLN (▲), microparticles (◆) and control (●) ($n = 3$ except 100 nm, 60 min is $n = 1$ (SEM not shown) and 400 nm, 60 min is $n = 2$). Modified with permission from Ref. 141.

3.3. *Solid lipid particles in pulmonary drug delivery*

Pulmonary drug delivery is used to deliver medicines directly to the lung through the respiratory system. It is an efficient administration route for combating lung diseases by targeting a single drug (or a drug combination) to the site of action. However, inhaled medicines and particles may undergo natural clearance, resulting in a short residence time in the airways[160]. Carriers providing sustained drug release with relatively long residence times in the respiratory tract can improve therapeutic outcomes of inhaled medicines by gradually releasing the drug locally and moderate the drug peaks to reduce toxicity[161−163]. The deposition and accumulation of particles in the lungs and their clearance from the lungs depend on particles' aerodynamic diameters and the breathing patterns[164,165]. Inhalation of nanoparticles with sizes of about 800 nm resulted in a longer drug retention in the lungs than SLNs with smaller nanoparticle sizes ranging from 200 to 400 nm[166]. The major nanoparticle clearance mechanisms in the lungs are phagocytosis clearance by macrophages and mucociliary clearance, where nanoparticles are trapped in the airway surface liquid and coughed up. Nanoparticles are less phagocytized by alveolar macrophages and can lead to deep lung deposition with potentials for lung cancer therapy[162,167,168].

Labeled SLNs have been used to investigate the deposition and clearance of lipid nanocarriers in the lungs. Among these investigations, lipid nanoparticles with sizes around 200 nm, prepared by using glyceryl dibehenate as a lipid phase and Tween 80 as an emulsifier, were labelled by incubation with ^{99m}Tc aqueous solution[169]. The results of post-inhalation image analysis and accumulation of radioactivity in different organ samples showed a strong deposition of SLNs in the lungs of rats[169]. The highest activity counting was observed in the lungs as compared to other organ samples collected 4 h after inhalation[169], suggesting the retention of SLNs in the lungs. A lipophilic florescent dye, DID-oil, was also used to track pulmonary deposition of solid lipid nanocarriers of celecoxib with nanoparticle sizes around 220 nm[170]. These nanoparticles were prepared by using a mixture of glyceryl dibehenate and medium-chain triglycerides as a lipid phase and sodium taurocholate as an emulsifier. Around 78% of the celecoxib-dose was detected in the lungs of mice after 30 min of nebulization, and the retention of the nanoparticles led to a constant drug concentration in the lungs for 2 h[170]. The deposition of nanoparticles in the lungs was confirmed by confocal observations with images of the lungs collected at 0.5 and 4 h after nebulization of the formulation in mice[170]. Enhanced antitumor activity was observed when SLNs were evaluated for pulmonary co-delivery of anticancer drugs and siRNA[171]. The lung tumor size was significantly reduced after inhalation of drug-loaded lipid nanoparticles with sizes about 110 nm, prepared using a mixture of glyceryl palmitostearate and squalene as a lipid phase and phospholipids and Tween 80 as emulsifiers[171]. The accumulation of lipid nanoparticles in the lungs and other organs after an inhalation or an intravenous treatment was investigated after labeling with Cy5.5[171]. It was reported on the presence of 83% of the nanoparticles in the lungs and 13% in the liver 24 h of post-inhalation, whereas 23% and 59% of the nanoparticles exist in the lungs and the liver, respectively, after intravenous treatment[171]. This study confirms the prolonged retention time of solid lipid

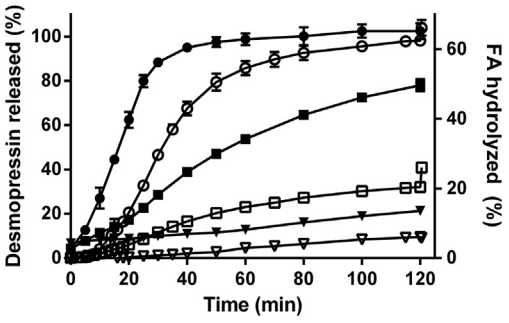

Figure 6 Release of desmopressin during *in vitro* lipolysis (closed symbols) and amount of hydrolyzed fatty acids (open symbols) from triglyceride (TG) particles. ●, TG14 particles; ■, TG16 particles; ▼, TG18 particles. Data are expressed as mean ± SD ($n = 3$). Reproduced with permission from Ref. 126.

nanocarriers in the lungs and the advantage of using SLNs as platforms with sustained delivery of drugs for the treatment of lung diseases. Aiming at an active targeting of drug-loaded SLNs to alveolar macrophages *via* the mannose receptor-mediated mechanism, a mannose-based surfactant was recently used in the formation of rifampicin-loaded SLNs consisting of a lipid phase based on a mixture of palmitic acid and cholesteryl myristate[172]. Real-time fluorescence imaging in living animals showed a high retention of SLNs with less spreading in extra-pulmonary regions after intratracheal instillation in mice[172].

Drug-loaded SLNs can be transformed to microparticles for improving their long-term storage colloidal stability. Inhalable microparticles (4 μm) were prepared by co-spray drying of thymopentin-loaded SLNs with sizes around 150 nm and bulking agents mannitol and leucine. These drug-loaded SLNs were prepared by a double emulsion method in the presence of glyceryl monostearate and phosphatidylcholine as a lipid phase and poloxamer 188 as an emulsifier[161]. The spray drying process did not change the essential properties of the SLNs apart from an improved aerosolization efficiency. Pulmonary administration of the microparticles containing FITC-labeled drug-loaded SLNs resulted in a spot distribution of drug powders in the pulmonary alveolus of rats, and an improved bioavailability and therapeutic efficacy of thymopentin[161].

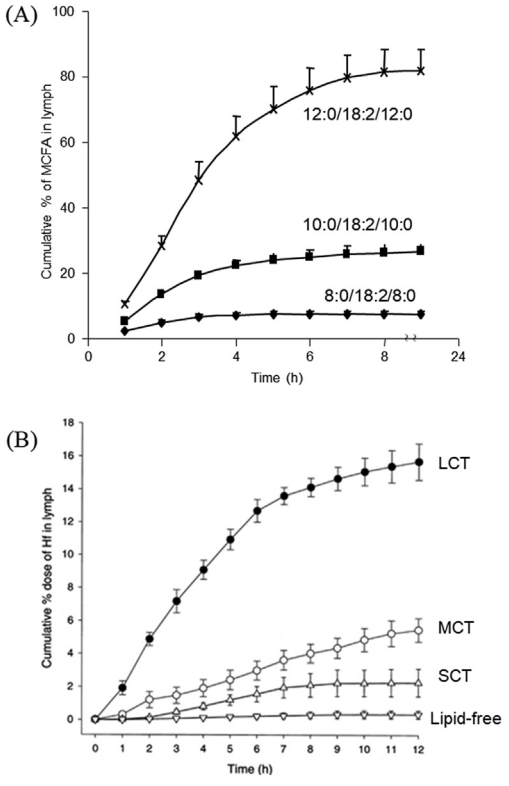

Figure 7 Lymphatic transport of lipids and halofantrine solubilised in lipids. (A) Cumulative lymphatic transport of fatty acids after intragastric administration of structured TG 8:0/18:2/8:0, 10:0/18:2/10:0, 12:0/18:2/12:0; (B) cumulative lymphatic transport of halofantrine after oral administration in different TG solutions. Reproduced with permission from Ref. 134.

4. Conclusions and perspective

ISAsomes and SLNs have shown great drug delivery application potentials for both systemic circulation and local applications. The fate of these lipid nanoparticles *in vivo* is affected by lipid composition and type, and different physicochemical properties including size characteristics and their surface properties, as well as the compositions of the biological fluids. This contribution presents different examples and highlights the main advantages of using cubosomes, hexosomes, and related nanoparticles as versatile platforms for drug delivery. Different examples on SLNs, particularly nanoformulations intended for oral and pulmonary drug delivery applications are also presented.

Despite the attractiveness of *ISAsomes*, mainly cubosomes and hexosomes, as nanocarriers for drug delivery applications, there are limited number of studies on their fate after *in vivo* administration. *In vitro* investigations, including cellular responses of these nano-self-assemblies were also conducted on a limited number of model cell lines. Most of investigations in the literature focused on the biophysical characterization and drug encapsulation of cubosomes and hexosomes. For gaining further information and exploring the potential clinical applications of these nano-self-assemblies, future investigations should focus on combining relevant *in vitro*/*in vivo* evaluations with biophysical experiments, and gaining further insights into their drug release and encapsulation properties. Taking into account the attractiveness of these nano-self-assemblies in the development of drug nanocarriers, we expect an increase in the number of reports in the literature on their *in vivo* fates following different administration routes within the next few years. It is a multidisciplinary research area and therefore, important to involve scientists from different backgrounds. It is also important to initiate more collaborative industry-academia research projects.

Oral administration of SLNs is often leading to improved bioavailability and enhanced efficacy, it is evident that degradation of SLNs in the gastrointestinal tract occurs during the process of drug release and absorption. SLNs containing long-chain fatty acids can facilitate formation of chylomicrons and lymphatic transport of lipophilic drugs. Enhanced accumulation of these nanoparticles with longer residence times in the lungs after inhalation improves therapeutic outcomes by gradually releasing the drug locally. Even though future applications of SLNs in

sustained oral drug delivery could be limited due to their short residence times in the gastrointestinal tract, the application potentials of these nanoparticles in sustained topical drug delivery as well as targeting to special tissues and organs could be further explored.

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Author contributions

Anan Yaghmur and Huiling Mu wrote, revised the manuscript, and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. *Pharm Res (N Y)* 2006;**23**:1417−50.
2. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. *Faseb J* 2005;**19**:311−30.
3. Wibroe PP, Ahmadvand D, Oghabian MA, Yaghmur A, Moghimi SM. An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil®, Caelyx®, DOXOrubicin, and SinaDoxosome. *J Control Release* 2016;**221**:1−8.
4. Garcia-Pinel B, Porras-Alcala C, Ortega-Rodriguez A, Sarabia F, Prados J, Melguizo C, et al. Lipid-based nanoparticles: application and recent advances in cancer treatment. *Nanomaterials* 2019;**9**:638.
5. Bor G, Mat Azmi ID, Yaghmur A. Nanomedicines for cancer therapy: current status, challenges and future prospects. *Ther Deliv* 2019;**10**:113−32.
6. Azmi ID, Moghimi SM, Yaghmur A. Cubosomes and hexosomes as versatile platforms for drug delivery. *Ther Deliv* 2015;**6**:1347−64.
7. Steichen SD, Caldorera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. *Eur J Pharmaceut Sci* 2013;**48**:416−27.
8. Murgia S, Biffi S, Mezzenga R. Recent advances of non-lamellar lyotropic liquid crystalline nanoparticles in nanomedicine. *Curr Opin Colloid Interface Sci* 2020;**48**:28−39.
9. Souto EB, Baldim I, Oliveira WP, Rao R, Yadav N, Gama FM, et al. SLN and NLC for topical, dermal, and transdermal drug delivery. *Expet Opin Drug Deliv* 2020;**17**:357−77.
10. Yingchoncharoen P, Kalinowski DS, Richardson DR. Lipid-based drug delivery systems in cancer therapy: what is available and what is yet to come. *Pharmacol Rev* 2016;**68**:701−87.
11. Sastri KT, Radha GV, Pidikiti S, Vajjhala P. Solid lipid nanoparticles: preparation techniques, their characterization, and an update on recent studies. *J Appl Pharmaceut Sci* 2020;**10**:126−41.
12. Pucek A, Tokarek B, Waglewska E, Bazylińska U. Recent advances in the structural design of photosensitive agent formulations using "soft" colloidal nanocarriers. *Pharmaceutics* 2020;**12**:587.
13. Mahant S, Rao R, Souto EB, Nanda S. Analytical tools and evaluation strategies for nanostructured lipid carrier based topical delivery systems. *Expet Opin Drug Deliv* 2020;**17**:963−92.
14. Tapeinos C, Battaglini M, Ciofani G. Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. *J Control Release* 2017;**264**:306−32.
15. Yaghmur A, Glatter O. Characterization and potential applications of nanostructured aqueous dispersions. *Adv Colloid Interface Sci* 2009;**147−48**:333−42.
16. Gordillo-Galeano A, Mora-Huertas CE. Solid lipid nanoparticles and nanostructured lipid carriers: a review emphasizing on particle structure and drug release. *Eur J Pharm Biopharm* 2018;**133**:285−308.
17. Glatter O, Salentinig S. Inverting structures: from micelles *via* emulsions to internally self-assembled water- and oil-continuous nanocarriers. *Curr Opin Colloid Interface Sci* 2020;**49**:82−93.
18. Khosa A, Reddi S, Saha RN. Nanostructured lipid carriers for site-specific drug delivery. *Biomed Pharmacother* 2018;**103**:598−613.
19. Paliwal R, Paliwal SR, Kenwat R, Kurmi BD, Sahu MK. Solid lipid nanoparticles: a review on recent perspectives and patents. *Expert Opin Ther Pat* 2020;**30**:179−94.
20. Angelova A, Garamus VM, Angelov B, Tian Z, Li Y, Zou A. Advances in structural design of lipid-based nanoparticle carriers for delivery of macromolecular drugs, phytochemicals and anti-tumor agents. *Adv Colloid Interface Sci* 2017;**249**:331−45.
21. Yaghmur A, Al-Hosayni S, Amenitsch H, Salentinig S. Structural investigation of bulk and dispersed inverse lyotropic hexagonal liquid crystalline phases of eicosapentaenoic acid monoglyceride. *Langmuir* 2017;**33**:14045−57.
22. Shao X, Bor G, Al-Hosayni S, Salentinig S, Yaghmur A. Structural characterization of self-assemblies of new omega-3 lipids: docosahexaenoic acid and docosapentaenoic acid monoglycerides. *Phys Chem Chem Phys* 2018;**20**:23928−41.
23. Gustafsson J, Ljusberg-Wahren H, Almgren M, Larsson K. Submicron particles of reversed lipid phases in water stabilized by a nonionic amphiphilic polymer. *Langmuir* 1997;**13**:6964−71.
24. Larsson K. Lyotropic liquid crystals and their dispersions relevant in foods. *Curr Opin Colloid Interface Sci* 2009;**14**:16−20.
25. de Campo L, Yaghmur A, Sagalowicz L, Leser ME, Watzke H, Glatter O. Reversible phase transitions in emulsified nanostructured lipid systems. *Langmuir* 2004;**20**:5254−61.
26. Yaghmur A, de Campo L, Sagalowicz L, Leser ME, Glatter O. Emulsified microemulsions and oil-containing liquid crystalline phases. *Langmuir* 2005;**21**:569−77.
27. Dong YD, Larson I, Hanley T, Boyd BJ. Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin E acetate and F127 polymer on liquid crystal nanostructure. *Langmuir* 2006;**22**:9512−8.
28. Nakano M, Teshigawara T, Sugita A, Leesajakul W, Taniguchi A, Kamo T, et al. Dispersions of liquid crystalline phases of the monoolein/oleic acid/Pluronic F127 system. *Langmuir* 2002;**18**:9283−8.
29. Gontsarik M, Buhmann MT, Yaghmur A, Ren Q, Maniura-Weber K, Salentinig S. Antimicrobial peptide-driven colloidal transformations in liquid-crystalline nanocarriers. *J Phys Chem Lett* 2016;**7**:3482−6.
30. Helvig SY, Andersen H, Antopolsky M, Airaksinen AJ, Urtti A, Yaghmur A, et al. Hexosome engineering for targeting of regional lymph nodes. *Materialia* 2020;**11**:100705.
31. Yaghmur A, Rappolt M, Jonassen ALU, Schmitt M, Larsen SW. In situ monitoring of the formation of lipidic non-lamellar liquid crystalline depot formulations in synovial fluid. *J Colloid Interface Sci* 2021;**582**:773−81.
32. Azmi ID, Wibroe PP, Wu LP, Kazem AI, Amenitsch H, Moghimi SM, et al. A structurally diverse library of safe-by-design citrem-phospholipid lamellar and non-lamellar liquid crystalline nano-assemblies. *J Control Release* 2016;**239**:1−9.
33. Azmi ID, Wu L, Wibroe PP, Nilsson C, Ostergaard J, Sturup S, et al. Modulatory effect of human plasma on the internal nanostructure and size characteristics of liquid-crystalline nanocarriers. *Langmuir* 2015;**31**:5042−9.
34. Azmi IDM, Ostergaard J, Sturup S, Gammelgaard B, Urtti A, Moghimi SM, et al. Cisplatin encapsulation generates morphologically different multicompartments in the internal nanostructures of nonlamellar liquid-crystalline self-assemblies. *Langmuir* 2018;**34**:6570−81.

35. Anderluzzi G, Lou G, Su Y, Perrie Y. Scalable manufacturing processes for solid lipid nanoparticles. *Pharm Nanotechnol* 2019;**7**:444−59.
36. Prajapati R, Salentinig S, Yaghmur A. Temperature triggering of kinetically trapped self-assemblies in citrem-phospholipid nanoparticles. *Chem Phys Lipids* 2018;**216**:30−8.
37. Xia H, Seah Y, Liu Y, Wang W, Toh AG, Wang Z. Anti-solvent precipitation of solid lipid nanoparticles using a microfluidic oscillator mixer. *Microfluid Nanofluidics* 2015;**19**:283−90.
38. Khaliqi K, Ghazal A, Azmi IDM, Amenitsch H, Mortensen K, Salentinig S, et al. Direct monitoring of lipid transfer on exposure of citrem nanoparticles to an ethanol solution containing soybean phospholipids by combining synchrotron SAXS with microfluidics. *Analyst* 2017;**142**:3118−26.
39. Yaghmur A, Ghazal A, Ghazal R, Dimaki M, Svendsen WE. A hydrodynamic flow focusing microfluidic device for the continuous production of hexosomes based on docosahexaenoic acid monoglyceride. *Phys Chem Chem Phys* 2019;**21**:13005−13.
40. Kim H, Sung J, Chang Y, Alfeche A, Leal C. Microfluidics synthesis of gene silencing cubosomes. *ACS Nano* 2018;**12**:9196−205.
41. Ilhan-Ayisigi E, Yaldiz B, Bor G, Yaghmur A, Yesil-Celiktas O. Advances in microfluidic synthesis and coupling with synchrotron SAXS for continuous production and real-time structural characterization of nano-self-assemblies. *Colloid Surface B* 2021;**201**:111633. Available from: https://doi.org/10.1016/j.colsurfb.2021.111633.
42. Yaghmur A, de Campo L, Sagalowicz L, Leser ME, Glatter O. Control of the internal structure of MLO-based isasomes by the addition of diglycerol monooleate and soybean phosphatidylcholine. *Langmuir* 2006;**22**:9919−27.
43. Yaghmur A, de Campo L, Salentinig S, Sagalowicz L, Leser ME, Glatter O. Oil-loaded monolinolein-based particles with confined inverse discontinuous cubic structure (Fd3m). *Langmuir* 2006;**22**:517−21.
44. Angelova A, Angelov B, Mutafchieva R, Lesieur S, Couvreur P. Self-assembled multicompartment liquid crystalline lipid carriers for protein, peptide, and nucleic acid drug delivery. *Acc Chem Res* 2011;**44**:147−56.
45. Zhai J, Fong C, Tran N, Drummond CJ. Non-lamellar lyotropic liquid crystalline lipid nanoparticles for the next generation of nanomedicine. *ACS Nano* 2019;**13**:6178−206.
46. Tan A, Hong L, Du JD, Boyd BJ. Self-assembled nanostructured lipid systems: is there a link between structure and cytotoxicity?. *Adv Sci* 2019;**6**:1801223.
47. Helvig SY, Woythe L, Pham S, Bor G, Andersen H, Moghimi SM, et al. A structurally diverse library of glycerol monooleate/oleic acid non-lamellar liquid crystalline nanodispersions stabilized with nonionic methoxypoly (ethylene glycol)(mPEG)-lipids showing variable complement activation properties. *J Colloid Interface Sci* 2021;**582**:906−17.
48. Salentinig S, Yaghmur A, Guillot S, Glatter O. Preparation of highly concentrated nanostructured dispersions of controlled size. *J Colloid Interface Sci* 2008;**326**:211−20.
49. Hempt C, Gontsarik M, Buerki-Thurnherr T, Hirsch C, Salentinig S. Nanostructure generation during milk digestion in presence of cell culture models simulating the small intestine. *J Colloid Interface Sci* 2020;**574**:430−40.
50. Salentinig S, Phan S, Hawley A, Boyd BJ. Self-assembly structure formation during the digestion of human breast milk. *Angew Chem Int Ed* 2015;**54**:1600−3.
51. Salentinig S, Amenitsch H, Yaghmur A. In situ monitoring of nanostructure formation during the digestion of mayonnaise. *ACS Omega* 2017;**2**:1441−6.
52. Yaghmur A, Lotfi S, Ariabod SA, Bor G, Gontsarik M, Salentinig S. Internal lamellar and inverse hexagonal liquid crystalline phases during the digestion of krill and astaxanthin oil-in-water emulsions. *Front Bioeng Biotechnol* 2019;**7**:384.
53. Salentinig S. Supramolecular structures in lipid digestion and implications for functional food delivery. *Curr Opin Colloid Interface Sci* 2019;**39**:190−201.
54. Yaghmur A. Nanoencapsulation of food ingredients by cubosomes and hexosomes. In: Jafari SM, editor. *Lipid-based nanostructures for food encapsulation purposes*, vol. 2, chap 12. Elsevier Book Series; 2019. p. 483−522.
55. Rosa M, Rosa Infante M, Miguel MdG, Lindman B. Spontaneous formation of vesicles and dispersed cubic and hexagonal particles in amino acid-based catanionic surfactant systems. *Langmuir* 2006;**22**:5588−96.
56. Spicer PT, Hayden KL, Lynch ML, Ofori-Boateng A, Burns JL. Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). *Langmuir* 2001;**17**:5748−56.
57. Chong JYT, Mulet X, Waddington LJ, Boyd BJ, Drummond CJ. Steric stabilisation of self-assembled cubic lyotropic liquid crystalline nanoparticles: high throughput evaluation of triblock polyethylene oxide-polypropylene oxide-polyethylene oxide copolymers. *Soft Matter* 2011;**7**:4768−77.
58. Nilsson C, Edwards K, Eriksson J, Larsen SW, Ostergaard J, Larsen C, et al. Characterization of oil-free and oil-loaded liquid-crystalline particles stabilized by negatively charged stabilizer citrem. *Langmuir* 2012;**28**:11755−66.
59. Wibroe PP, Mat Azmi ID, Nilsson C, Yaghmur A, Moghimi SM. Citrem modulates internal nanostructure of glyceryl monooleate dispersions and bypasses complement activation: towards development of safe tunable intravenous lipid nanocarriers. *Nanomedicine* 2015;**11**:1909−14.
60. Nilsson C, Ostergaard J, Larsen SW, Larsen C, Urtti A, Yaghmur A. PEGylation of phytantriol-based lyotropic liquid crystalline particles–the effect of lipid composition, PEG chain length, and temperature on the internal nanostructure. *Langmuir* 2014;**30**:6398−407.
61. Hinton TM, Grusche F, Acharya D, Shukla R, Bansal V, Waddington LJ, et al. Bicontinuous cubic phase nanoparticle lipid chemistry affects toxicity in cultured cells. *Toxicol Res* 2014;**3**:11−22.
62. Murgia S, Falchi AM, Mano M, Lampis S, Angius R, Carnerup AM, et al. Nanoparticles from lipid-based liquid crystals: emulsifier influence on morphology and cytotoxicity. *J Phys Chem B* 2010;**114**:3518−25.
63. Fornasier M, Biffi S, Bortot B, Macor P, Manhart A, Wurm FR, et al. Cubosomes stabilized by a polyphosphoester-analog of Pluronic F127 with reduced cytotoxicity. *J Colloid Interface Sci* 2020;**580**:286−97.
64. Zabara M, Senturk B, Gontsarik M, Ren Q, Rottmar M, Maniura-Weber K, et al. Multifunctional nano-biointerfaces: cytocompatible antimicrobial nanocarriers from stabilizer-free cubosomes. *Adv Funct Mater* 2019;**29**:1904007.
65. Boyd BJ, Dong YD, Rades T. Nonlamellar liquid crystalline nanostructured particles: advances in materials and structure determination. *J Liposome Res* 2009;**19**:12−28.
66. Helvig S, Azmi IDM, Moghimi SM, Yaghmur A. Recent advances in cryo-TEM imaging of soft lipid nanoparticles. *Aims Biophys* 2015;**2**:116−30.
67. Yaghmur A, Laggner P, Almgren M, Rappolt M. Self-assembly in monoelaidin aqueous dispersions: direct vesicles to cubosomes transition. *PLoS One* 2008;**3**:e3747.
68. Yaghmur A, Sartori B, Rappolt M. Self-assembled nanostructures of fully hydrated monoelaidin-elaidic acid and monoelaidin-oleic acid systems. *Langmuir* 2012;**28**:10105−19.
69. Prajapati R, Gontsarik M, Yaghmur A, Salentinig S. pH-Responsive nano-self-assemblies of the anticancer drug 2-hydroxyoleic acid. *Langmuir* 2019;**35**:7954−61.
70. Prajapati R, Larsen SW, Yaghmur A. Citrem-phosphatidylcholine nano-self-assemblies: solubilization of bupivacaine and its role in triggering colloidal transition from vesicles to cubosomes and hexosomes. *Phys Chem Chem Phys* 2019;**21**:15142−50.

71. Kluzek M, Tyler AII, Wang S, Chen R, Marques CM, Thalmann F, et al. Influence of a pH-sensitive polymer on the structure of monoolein cubosomes. *Soft Matter* 2017;**13**:7571−7.
72. Ghazal A, Gontsarik M, Kutter JP, Lafleur JP, Labrador A, Mortensen K, et al. Direct monitoring of calcium-triggered phase transitions in cubosomes using small-angle X-ray scattering combined with microfluidics. *J Appl Crystallogr* 2016;**49**:2005−14.
73. Yaghmur A, Sartori B, Rappolt M. The role of calcium in membrane condensation and spontaneous curvature variations in model lipidic systems. *Phys Chem Chem Phys* 2011;**13**:3115−25.
74. Yaghmur A, Rappolt M. Structural characterization of lipidic systems under nonequilibrium conditions. *Eur Biophys J* 2012;**41**:831−40.
75. Yaghmur A, Laggner P, Sartori B, Rappolt M. Calcium triggered L alpha-H2 phase transition monitored by combined rapid mixing and time-resolved synchrotron SAXS. *PLoS One* 2008;**3**:e2072.
76. Almgren M, Edwards K, Karlsson G. Cryo transmission electron microscopy of liposomes and related structures. *Colloid Surface* 2000;**174**:3−21.
77. Sagalowicz L, Michel M, Adrian M, Frossard P, Rouvet M, Watzke HJ, et al. Crystallography of dispersed liquid crystalline phases studied by cryo-transmission electron microscopy. *J Microsc-Oxford* 2006;**221**:110−21.
78. Yaghmur A, Tran BV, Moghimi SM. Non-lamellar liquid crystalline nanocarriers for thymoquinone encapsulation. *Molecules* 2020;**25**:16.
79. Barauskas J, Johnsson M, Tiberg F. Self-assembled lipid superstructures: beyond vesicles and liposomes. *Nano Lett* 2005;**5**:1615−9.
80. Tajik-Ahmadabad B, Chollet L, White J, Separovic F, Polyzos A. Metallo-cubosomes: zinc-functionalized cubic nanoparticles for therapeutic nucleotide delivery. *Mol Pharm* 2019;**16**:978−86.
81. Rakotoarisoa M, Angelov B, Garamus VM, Angelova A. Curcumin-and fish oil-loaded spongosome and cubosome nanoparticles with neuroprotective potential against H_2O_2-induced oxidative stress in differentiated human SH-SY5Y cells. *ACS Omega* 2019;**4**:3061−73.
82. Faria AR, Silvestre OF, Maibohm C, Adao RMR, Silva BFB, Nieder JB. Cubosome nanoparticles for enhanced delivery of mitochondria anticancer drug elesclomol and therapeutic monitoring *via* sub-cellular NAD(P)H multi-photon fluorescence lifetime imaging. *Nano Res* 2019;**12**:991−8.
83. Khan S, Jain P, Jain S, Jain R, Bhargava S, Jain A. Topical delivery of erythromycin through cubosomes for acne. *Pharm Nanotechnol* 2018;**6**:38−47.
84. Barriga HMG, Holme MN, Stevens MM. Cubosomes; the next generation of smart lipid nanoparticles?. *Angew Chem Int Ed Engl* 2019;**58**:2958−78.
85. Gontsarik M, Yaghmur A, Ren Q, Maniura-Weber K, Salentinig S. From structure to function: pH-Switchable antimicrobial nano-self-assemblies. *ACS Appl Mater Interfaces* 2019;**11**:2821−9.
86. Gontsarik M, Mohammadtaheri M, Yaghmur A, Salentinig S. pH-Triggered nanostructural transformations in antimicrobial peptide/oleic acid self-assemblies. *Biomater Sci* 2018;**6**:803−12.
87. Mertins O, Mathews PD, Angelova A. Advances in the design of pH-sensitive cubosome liquid crystalline nanocarriers for drug delivery applications. *Nanomaterials* 2020;**10**:963.
88. Gontsarik M, Yaghmur A, Salentinig S. Dispersed liquid crystals as pH-adjustable antimicrobial peptide nanocarriers. *J Colloid Interface Sci* 2021;**583**:672−82.
89. Nguyen T-H, Hanley T, Porter CJ, Boyd BJ. Nanostructured liquid crystalline particles provide long duration sustained-release effect for a poorly water soluble drug after oral administration. *J Control Release* 2011;**153**:180−6.
90. Puglia C, Cardile V, Panico AM, Crasci L, Offerta A, Caggia S, et al. Evaluation of monooleine aqueous dispersions as tools for topical administration of curcumin: characterization, *in vitro* and *ex-vivo* studies. *J Pharmacol Sci* 2013;**102**:2349−61.
91. Tran N, Mulet X, Hawley AM, Hinton TM, Mudie ST, Muir BW, et al. Nanostructure and cytotoxicity of self-assembled monoolein-capric acid lyotropic liquid crystalline nanoparticles. *RSC Adv* 2015;**5**:26785−95.
92. Shen HH, Crowston JG, Huber F, Saubern S, McLean KM, Hartley PG. The influence of dipalmitoyl phosphatidylserine on phase behaviour of and cellular response to lyotropic liquid crystalline dispersions. *Biomaterials* 2010;**31**:9473−81.
93. Li Y, Angelova A, Hu F, Garamus VM, Peng C, Li N, et al. pH Responsiveness of hexosomes and cubosomes for combined delivery of brucea javanica oil and doxorubicin. *Langmuir* 2019;**35**:14532−42.
94. Ramalheiro A, Paris JL, Silva BF, Pires LR. Rapidly dissolving microneedles for the delivery of cubosome-like liquid crystalline nanoparticles with sustained release of rapamycin. *Int J Pharm* 2020:119942.
95. Flak DK, Adamski V, Nowaczyk G, Szutkowski K, Synowitz M, Jurga S, et al. AT101-loaded cubosomes as an alternative for improved glioblastoma therapy. *Int J Nanomed* 2020;**15**:7415−31.
96. Bakr MM, Shukr MH, ElMeshad AN. In situ hexosomal gel as a promising tool to ameliorate the transnasal brain delivery of vinpocetine: central composite optimization and *in vivo* biodistribution. *J Pharmacol Sci* 2020;**109**:2213−23.
97. Wu H, Li J, Zhang Q, Yan X, Guo L, Gao X, et al. A novel small Odorranalectin-bearing cubosomes: preparation, brain delivery and pharmacodynamic study on amyloid-β25−35-treated rats following intranasal administration. *Eur J Pharm Biopharm* 2012;**80**:368−78.
98. Silvestrin AVP, Caron AL, Viegas J, Praça FG, Bentley MVLB. Advances in lyotropic liquid crystal systems for skin drug delivery. *Expet Opin Drug Deliv* 2020;**17**:1781−805.
99. de Carvalho Vicentini FTM, Depieri LV, Polizello ACM, Del Ciampo JO, Spadaro ACC, Fantini MC, et al. Liquid crystalline phase nanodispersions enable skin delivery of siRNA. *Eur J Pharm Biopharm* 2013;**83**:16−24.
100. Kang M, Leal C. Soft nanostructured films for actuated surface-based siRNA delivery. *Adv Funct Mater* 2016;**26**:5610−20.
101. Li Y, Angelova A, Liu J, Garamus VM, Li N, Drechsler M, et al. In situ phase transition of microemulsions for parenteral injection yielding lyotropic liquid crystalline carriers of the antitumor drug bufalin. *Colloids Surf, B* 2019;**173**:217−25.
102. Mei L, Xie Y, Huang Y, Wang B, Chen J, Quan G, et al. Injectable in situ forming gel based on lyotropic liquid crystal for persistent postoperative analgesia. *Acta Biomater* 2018;**67**:99−110.
103. Yaghmur A, Larsen SW, Schmitt M, Ostergaard J, Larsen C, Jensen H, et al. In situ characterization of lipidic bupivacaine-loaded formulations. *Soft Matter* 2011;**7**:8291−5.
104. Yaghmur A, Rappolt M, Ostergaard J, Larsen C, Larsen SW. Characterization of bupivacaine-loaded formulations based on liquid crystalline phases and microemulsions: the effect of lipid composition. *Langmuir* 2012;**28**:2881−9.
105. Yaghmur A, Rappolt M, Larsen SW. In situ forming drug delivery systems based on lyotropic liquid crystalline phases: structural characterization and release properties. *J Drug Deliv Sci Tec* 2013;**23**:325−32.
106. von Halling Laier C, Gibson B, Moreno JAS, Rades T, Hook S, Nielsen LH, et al. Microcontainers for protection of oral vaccines, *in vitro* and *in vivo* evaluation. *J Control Release* 2019;**294**:91−101.
107. Jin X, Zhang ZH, Li SL, Sun E, Tan XB, Song J, et al. A nanostructured liquid crystalline formulation of 20 (S)-protopanaxadiol with improved oral absorption. *Fitoterapia* 2013;**84**:64−71.
108. Otte A, Soh B-K, Yoon G, Park K. Liquid crystalline drug delivery vehicles for oral and IV/subcutaneous administration of poorly soluble (and soluble) drugs. *Int J Pharm* 2018;**539**:175−83.
109. Swarnakar NK, Thanki K, Jain S. Bicontinuous cubic liquid crystalline nanoparticles for oral delivery of doxorubicin: implications on bioavailability, therapeutic efficacy, and cardiotoxicity. *Pharm Res (N Y)* 2014;**31**:1219−38.
110. Yang Z, Chen M, Yang M, Chen J, Fang W, Xu P. Evaluating the potential of cubosomal nanoparticles for oral delivery of amphotericin B in treating fungal infection. *Int J Nanomed* 2014;**9**:327.

111. Tran N, Bye N, Moffat BA, Wright DK, Cuddihy A, Hinton TM, et al. Dual-modality NIRF-MRI cubosomes and hexosomes: high throughput formulation and *in vivo* biodistribution. *Mater Sci Eng C Mater Biol Appl* 2017;**71**:584−93.
112. Biffi S, Andolfi L, Caltagirone C, Garrovo C, Falchi AM, Lippolis V, et al. Cubosomes for *in vivo* fluorescence lifetime imaging. *Nanotechnology* 2017;**28**:055102.
113. Bye N, Hutt OE, Hinton TM, Acharya DP, Waddington LJ, Moffat BA, et al. Nitroxide-loaded hexosomes provide MRI contrast *in vivo*. *Langmuir* 2014;**30**:8898−906.
114. Jain V, Swarnakar NK, Mishra PR, Verma A, Kaul A, Mishra AK, et al. Paclitaxel loaded PEGylated gleceryl monooleate based nanoparticulate carriers in chemotherapy. *Biomaterials* 2012;**33**:7206−20.
115. Zhai J, Tan F, Luwor R, Srinivasa Reddy T, Ahmed N, Drummond CJ, et al. *In vitro* and *in vivo* toxicity, and biodistribution of paclitaxel-loaded cubosomes as a drug delivery nanocarrier: a case study using an A431 skin cancer xenograft model. *ACS Applied Bio Materials* 2020;**3**:4198−207.
116. Tian Y, Li JC, Zhu JX, Zhu N, Zhang HM, Liang L, et al. Folic acid-targeted etoposide cubosomes for theranostic application of cancer cell imaging and therapy. *Med Sci Mon Int Med J Exp Clin Res* 2017;**23**:2426−35.
117. Grislain L, Couvreur P, Lenaerts V, Roland M, Deprez-Decampeneere D, Speiser P. Pharmacokinetics and distribution of a biodegradable drug-carrier. *Int J Pharm* 1983;**15**:335−45.
118. Nilsson C, Barrios-Lopez B, Kallinen A, Laurinmaki P, Butcher SJ, Raki M, et al. SPECT/CT imaging of radiolabeled cubosomes and hexosomes for potential theranostic applications. *Biomaterials* 2013;**34**:8491−503.
119. Moghimi SM. Modulation of lymphatic distribution of subcutaneously injected poloxamer 407-coated nanospheres: the effect of the ethylene oxide chain configuration. *FEBS Lett* 2003;**545**:260.
120. Nasr M, Ghorab MK, Abdelazem A. *In vitro* and *in vivo* evaluation of cubosomes containing 5-fluorouracil for liver targeting. *Acta Pharm Sin B* 2015;**5**:79−88.
121. Liu ZG, Luo L, Zheng SS, Niu YL, Bo RN, Huang Y, et al. Cubosome nanoparticles potentiate immune properties of immunostimulants. *Int J Nanomed* 2016;**11**:3571−83.
122. Rizwan SB, McBurney WT, Young K, Hanley T, Boyd BJ, Rades T, et al. Cubosomes containing the adjuvants imiquimod and monophosphoryl lipid A stimulate robust cellular and humoral immune responses. *J Control Release* 2013;**165**:16−21.
123. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery — a review of the state of the art. *Eur J Pharm Biopharm* 2000;**50**:161−77.
124. Zhang QN, Yie GQ, Li Y, Yang QS, Nagai YT. Studies on the cyclosporin A loaded stearic acid nanoparticles. *Int J Pharm* 2000;**200**:153−9.
125. Mu HL, Holm R. Solid lipid nanocarriers in drug delivery: characterization and design. *Expet Opin Drug Deliv* 2018;**15**:771−85.
126. Christophersen PC, Zhang L, Mullertz A, Nielsen HM, Yang M, Mu H. Solid lipid particles for oral delivery of peptide and protein drugs II — the digestion of trilaurin protects desmopressin from proteolytic degradation. *Pharm Res (N Y)* 2014;**31**:2420−8.
127. Gamboa CK, Samir R, Wu C, Mu H. Solid lipid particles as drug carriers - effects of particle preparation methods and lipid excipients on particle characteristics. *Pharm Nanotechnol* 2018;**6**:124−32.
128. Ke PC, Lin S, Parak WJ, Davis TP, Caruso F. A decade of the protein corona. *ACS Nano* 2017;**11**:11773−6.
129. Peng Q, Liu JY, Zhang T, Zhang TX, Zhang CL, Mu HL. Digestive enzyme corona formed in the gastrointestinal tract and its impact on epithelial cell uptake of nanoparticles. *Biomacromolecules* 2019;**20**:1789−97.
130. Wiedenmann V, Oehlke K, van der Schaaf U, Schrader K, Karbstein HP. Heat stability of differently stabilized solid lipid nanoparticles in the presence of excess bulk phase protein. *Food Biophys* 2019;**14**:393−402.
131. Maretti E, Rustichelli C, Gualtieri ML, Costantino L, Siligardi C, Miselli P, et al. The impact of lipid corona on rifampicin intramacrophagic transport using inhaled solid lipid nanoparticles surface-decorated with a mannosylated surfactant. *Pharmaceutics* 2019;**11**:508.
132. Peng Q, Mu HL. The potential of protein-nanomaterial interaction for advanced drug delivery. *J Control Release* 2016;**225**:121−32.
133. Mu H, Hoy CE. The digestion of dietary triacylglycerols. *Prog Lipid Res* 2004;**43**:105−33.
134. Mu HL, Holm R, Mullertz A. Lipid-based formulations for oral administration of poorly water-soluble drugs. *Int J Pharm* 2013;**453**:215−24.
135. Zara GP, Bargoni A, Cavalli R, Fundaro A, Vighetto D, Gasco MR. Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats. *J Pharmacol Sci* 2002;**91**:1324−33.
136. Gaur PK, Mishra S, Bajpai M, Mishra A. Enhanced oral bioavailability of efavirenz by solid lipid nanoparticles: *in vitro* drug release and pharmacokinetics studies. *BioMed Res Int* 2014:363404.
137. Elbahwy IA, Ibrahim HM, Ismael HR, Kasem AA. Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles. *J Drug Deliv Sci Technol* 2017;**38**:78−89.
138. Shangguan MZ, Lu Y, Qi JP, Han J, Tian ZQ, Xie YC, et al. Binary lipids-based nanostructured lipid carriers for improved oral bioavailability of silymarin. *J Biomater Appl* 2014;**28**:887−96.
139. Liu Y, Wang L, Zhao YQ, He M, Zhang X, Niu MM, et al. Nanostructured lipid carriers versus microemulsions for delivery of the poorly water-soluble drug luteolin. *Int J Pharm* 2014;**476**:169−77.
140. Yu Q, Hu XW, Ma YH, Xie YC, Lu Y, Qi JP, et al. Lipids-based nanostructured lipid carriers (NLCs) for improved oral bioavailability of sirolimus. *Drug Deliv* 2016;**23**:1469−75.
141. Kaithwas V, Dora CP, Kushwah V, Jain S. Nanostructured lipid carriers of olmesartan medoxomil with enhanced oral bioavailability. *Colloids Surf, B* 2017;**154**:10−20.
142. Liu YJ, Salituro GM, Lee KJ, Bak A, Leung DH. Modulating drug release and enhancing the oral bioavailability of torcetrapib with solid lipid dispersion formulations. *AAPS PharmSciTech* 2015;**16**:1091−100.
143. Borkar N, Xia DN, Holm R, Gan Y, Mullertz A, Yang MS, et al. Investigating the correlation between *in vivo* absorption and *in vitro* release of fenofibrate from lipid matrix particles in biorelevant medium. *Eur J Pharmaceut Sci* 2014;**51**:204−10.
144. Christophersen PC, Zhang L, Yang M, Nielsen HM, Mullertz A, Mu H. Solid lipid particles for oral delivery of peptide and protein drugs I-Elucidating the release mechanism of lysozyme during lipolysis. *Eur J Pharm Biopharm* 2013;**2013**:473−80.
145. Holm R, Porsgaard T, Porter CJH, Hoy CE, Edwards GA, Mullertz A, et al. Lymphatic fatty acids in canine with pharmaceutical formulations containing structured triacylglycerols. *Eur J Lipid Sci Technol* 2006;**108**:714−22.
146. Dahan A, Hoffman A. Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs. *Eur J Pharmaceut Sci* 2005;**24**:381−8.
147. Caliph SM, Charman WN, Porter CJ. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J Pharmacol Sci* 2000;**89**:1073−84.
148. Mu HL, Hoy CE. Intestinal absorption of specific structured triacylglycerols. *J Lipid Res* 2001;**42**:792−8.
149. Mu HL, Hoy CE. Distribution of medium-chain FA in different lipid classes after administration of specific structured TAG in rats. *Lipids* 2002;**37**:329−31.
150. Cai S, Yang QH, Bagby TR, Forrest ML. Lymphatic drug delivery using engineered liposomes and solid lipid nanoparticles. *Adv Drug Deliv Rev* 2011;**63**:901−8.

151. Singh I, Swami R, Khan W, Sistla R. Lymphatic system: a prospective area for advanced targeting of particulate drug carriers. *Expet Opin Drug Deliv* 2014;**11**:211−29.
152. Makwana V, Jain R, Patel K, Nivsarkar M, Joshi A. Solid lipid nanoparticles (SLN) of efavirenz as lymph targeting drug delivery system: elucidation of mechanism of uptake using chylomicron flow blocking approach. *Int J Pharm* 2015;**495**:439−46.
153. Beloqui A, Solinis MA, Delgado A, Evora C, Isla A, Rodriguez-Gascon A. Fate of nanostructured lipid carriers (NLCs) following the oral route: design, pharmacokinetics and biodistribution. *J Microencapsul* 2014;**31**:1−8.
154. Bargoni A, Cavalli R, Caputo O, Fundaro A, Gasco MR, Zara GP. Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. *Pharm Res (N Y)* 1998;**15**:745−50.
155. Beloqui A, Solinis MA, Gascon AR, del Pozo-Rodriguez A, des Rieux AD, Preat V. Mechanism of transport of saquinavir-loaded nanostructured lipid carriers across the intestinal barrier. *J Control Release* 2013;**166**:115−23.
156. Neves AR, Queiroz JF, Lima SAC, Figueiredo F, Fernandes R, Reis S. Cellular uptake and transcytosis of lipid-based nanoparticles across the intestinal barrier: relevance for oral drug delivery. *J Colloid Interface Sci* 2016;**463**:258−65.
157. Zhang ZW, Gao F, Bu HH, Xiao JS, Li YP. Solid lipid nanoparticles loading candesartan cilexetil enhance oral bioavailability: *in vitro* characteristics and absorption mechanism in rats. *Nanomedicine* 2012;**8**:740−7.
158. Wang T, Luo Y. Biological fate of ingested lipid-based nanoparticles: current understanding and future directions. *Nanoscale* 2019;**11**:11048−63.
159. Hu XW, Fan WF, Yu Z, Lu Y, Qi JP, Zhang J, et al. Evidence does not support absorption of intact solid lipid nanoparticles *via* oral delivery. *Nanoscale* 2016;**8**:7024−35.
160. Du J, Du P, Smyth HD. Hydrogels for controlled pulmonary delivery. *Ther Deliv* 2013;**4**:1293−305.
161. Li YZ, Sun X, Gong T, Liu J, Zuo JA, Zhang ZR. Inhalable microparticles as carriers for pulmonary delivery of thymopentin-loaded solid lipid nanoparticles. *Pharm Res* 2010;**27**:1977−86.
162. Weber S, Zimmer A, Pardeike J. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for pulmonary application: a review of the state of the art. *Eur J Pharm Biopharm* 2014;**86**:7−22.
163. Videira M, Almeida AJ, Fabra A. Preclinical evaluation of a pulmonary delivered paclitaxel-loaded lipid nanocarrier antitumor effect. *Nanomedicine* 2012;**8**:1208−15.
164. Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, et al. Large porous particles for pulmonary drug delivery. *Science* 1997;**276**:1868−71.
165. Wauthoz N, Amighi K. Phospholipids in pulmonary drug delivery. *Eur J Lipid Sci Technol* 2014;**116**:1114−28.
166. Zhao Y, Chang YX, Hu X, Liu CY, Quan LH, Liao YH. Solid lipid nanoparticles for sustained pulmonary delivery of Yuxingcao essential oil: preparation, characterization and *in vivo* evaluation. *Int J Pharm* 2017;**516**:364−71.
167. Abdelaziz HM, Gaber M, Abd-Elwakil MM, Mabrouk MT, Elgohary MM, Kamel NM, et al. Inhalable particulate drug delivery systems for lung cancer therapy: nanoparticles, microparticles, nanocomposites and nanoaggregates. *J Control Release* 2018;**269**:374−92.
168. Semmler-Behnke M, Takenaka S, Fertsch S, Wenk A, Seitz J, Mayer P, et al. Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent reentrainment onto airways epithelium. *Environ Health Perspect* 2007;**115**:728−33.
169. Videira MA, Botelho MF, Santos AC, Gouveia LF, de Lima JJP, Almeida AJ. Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. *J Drug Target* 2002;**10**:607−13.
170. Patlolla RR, Chougule M, Patel AR, Jackson T, Tata PNV, Singh M. Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. *J Control Release* 2010;**144**:233−41.
171. Taratula O, Kuzmov A, Shah M, Garbuzenko OB, Minko T. Nanostructured lipid carriers as multifunctional nanomedicine platform for pulmonary co-delivery of anticancer drugs and siRNA. *J Control Release* 2013;**171**:349−57.
172. Truzzi E, Nascimento TL, Iannuccelli V, Costantino L, Lima EM, Leo E, et al. *In vivo* biodistribution of respirable solid lipid nanoparticles surface-decorated with a mannose-based surfactant: a promising tool for pulmonary tuberculosis treatment?. *Nanomaterials* 2020;**10**:568.