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

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Received 4 November 2020; received in revised form 11 January 2021; accepted 18 January 2021

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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Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.02.013
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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\textsuperscript{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\textsuperscript{3,4}. They are particularly attractive for loading poorly water-soluble drugs that typically display limited bioavailability, poor pharmacokinetics, and adverse side effects\textsuperscript{7–9}. Surface engineering can also be employed through different surface manipulation strategies on lipid nanoparticles for active drug targeting\textsuperscript{10,11,12}.

Aside from liposomes, many efforts have been devoted in the utilization of emulsions, micellar solutions including micro-emulsions, solid lipid nanoparticles (SLNs), and non-lamellar liquid crystalline nanoparticles (mainly cubosomes and hexosomes) in the development of nanocarriers for drug delivery applications\textsuperscript{1,2,4,5,7–14}. Among these nanoparticulate, 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\textsuperscript{15}.

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\textsuperscript{8,9,11,15}. In SLNs, the internal architectures are solid crystalline phases\textsuperscript{9,11,12,16}; whereas cubosomes and hexosomes envelope internally non-lamellar liquid crystalline phases (inverse bicontinuous (Q\textsubscript{2}) and discontinuous hexagonal (H\textsubscript{2}) phases, respectively)\textsuperscript{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\textsuperscript{9,11,16,18}. 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 Pluronic and Tween 80 are among the most used stabilizers\textsuperscript{9,11,12,16,19}. However, cubosomes and hexosomes are generally produced from single amphiphiles (or amphiphile combinations) with high propensity to form inverse non-lamellar liquid crystalline phases at room and body temperatures, and typically stabilized with Pluronic F127\textsuperscript{20,21}. In their preparations, the most used amphiphiles include unsaturated monoglycerides, phytantriol, and their combinations with oils such as vitamin E and oleic acid\textsuperscript{22–31}. 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 IS\textsubscript{Asomes}, 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\textsuperscript{32–34}. Similar to non-lamellar liquid crystalline nanoparticles (having typically sizes in the range of 100–200 nm\textsuperscript{35–37}), 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\textsuperscript{14,16,18,35}. In addition to the typical low- and high-energy emulsification methods (including ultrasonication and high-pressure homogenization) for SLN, cubosome and hexosome preparations\textsuperscript{19,20,21,23,35}, other emulsification methods including modified high-pressure homogenization methods have been introduced for SLN production\textsuperscript{11,16,35}. There is also a growing interest in utilizing microfluidics for controlling the size and size distributions of these solid and non-lamellar liquid crystalline nanoparticles\textsuperscript{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 IS\textsubscript{Asomes} (Internally self-assembled somes), include cubosomes, hexosomes, micellar cubosomes, hexosomes, and emulsified microemulsions (EMEs)\textsuperscript{42–48}. These nano-self-assemblies have interior architectures of inverse bicontinuous cubic (Q\textsubscript{2}) phases, inverse discontinuous hexagonal (H\textsubscript{2}) and cubic Fd\textsubscript{d}m phases, and inverse microemulsions (water-in-oil microemulsions, L\textsubscript{2} phases), respectively\textsuperscript{49–54}. 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\textsuperscript{55–59}. It was proposed that they act as nanostructured carriers for facilitating the delivery of poorly water-soluble nutrients including vitamins\textsuperscript{60}.

2.1. Formation and characterization of IS\textsubscript{Asomes}

Alongside liposomes, the most investigated lipid nanoparticles for drug delivery and bio-imaging applications, there has been an increased interest in exploring IS\textsubscript{Asomes} (mainly cubosomes and hexosomes) as attractive versatile nanoplatforms for biomedical and pharmaceutical applications\textsuperscript{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\textsuperscript{6,15,17,20,54}. In the 1990s, Larsson and co-workers\textsuperscript{52} 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\textsuperscript{60,61}. Following a similar approach, known in literature as the bottom-up approach, it is possible to produce these nanoparticles by adding a suitable hydro trope, prior to the application of the low-energy emulsification method\textsuperscript{60}. 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
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\(^6,8,15,17,20,53\). 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\(^6,8,15,20,33,66\). 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\(^6,15,17,20,32,33,66\). In addition to SAXS and SANS static investigations\(^6,15,20,34,60–71\) 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\(^40,72–75\).

In addition, 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\(^6,34,47,66,77\). 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\(^30,32,34,47,78,79\). 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\(^670,78,81,89,90\). 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...
combining SAXS with cryo-TEM and DLS (or NTA) as mentioned above. 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. 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, opthalmic, and skin drug delivery, and their uses in the development of theranostic nanocarriers, the readers are directed to relevant reports and recent review articles. 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. They are attractive in the design of parenteral formulations with tunable nanostructures and sustained release properties.

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 (99mTc)-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.

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
release of various drugs [including 20(S)-protopanaxadiol, 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\(^{\text{C226}}\), 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\(^{\text{C226}}\), 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 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 \(^{99}\text{Tc}\)-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-distearyl-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.\(^{118}\).
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.

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.

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 P127-stabilized cubosomes and hexosomes as agents with dual MR-NIRF imaging properties. Through NIRF imaging, 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. 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. 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. 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. In addition to the investigated biodistribution of cubosomes and hexosomes, in vivo MR imaging indicated an enhanced contrast in the liver and spleen. An enhancement of MR contrast for in vivo imaging was also reported for nitroxide-loaded MO hexosomes. In another study, Jain et al. reported on radio-labeling of PEGylated non-lamellar liquid crystalline nanoparticles loaded with paclitaxel 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. 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, 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. 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.

Similar to other nanoparticles, 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 (99mTc) in the presence of 12-diamino-3,6,9-triazododecane (SpmTrien) as a chelating agent. In two reports, the synthesized 99mTc-SpmTrien-hexosomes were evaluated for in vivo imaging after subcutaneous (s.c.) administration to right flanks of healthy mice and footpads of healthy rats 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 99mTc-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. In the follow-up study, the injection of 99mTc-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. 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. 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.

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. 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. 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. 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.

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. The major excipients of the SLNs are solid lipids at room temperature, containing typically long-chain saturated fatty acids as the basic building blocks. 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. 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\cite{125,133}. 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 ($C_{\text{max}}$), and thereby an improved reduction in potential toxicity of drug substances. Delayed time to reach maximum drug concentration ($T_{\text{max}}$) has been reported in several pharmacokinetic studies\cite{124,138,139}, for example the $T_{\text{max}}$ for cyclosporine after oral administration of drug-loaded SLNs and microemulsion was 4.0 and 1.7 h, respectively\footnote{124}. Pharmacodynamic study has also shown a prolonged therapeutic effect after an oral administration of drug-loaded SLNs\cite{125,139}. Even though various in vivo studies have shown that SLNs can improve drug absorption and bioavailability\cite{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 $T_{\text{max}}$ 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\cite{142,143}. In these studies, the solid lipid nanocarriers led to a better bioavailability as compared to solid lipid microparticles\cite{132,143}. 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\cite{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)\footnote{143}. Additionally, drug release from lipid particles is affected by lipid excipient\cite{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)\footnote{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\cite{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)\footnote{134}. Both direct analysis of lymph samples collected from cannulated lymph duct in animals and following a chylomicon flow blocking approach have been used in studies of lymphatic transport of drugs and lipids\cite{145-146}. Different particulate drug carriers have been investigated for their potential in delivery of drugs via the intestinal lymphatic system\footnote{145}. Among these studies, lymphatic transport of efavirenz-loaded SLNs after an oral gavage was evaluated using both chylomicon flow blocking approach and lymph duct cannulated rat model\footnote{150}. 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\footnote{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\footnote{153}. In this study, the lipid nanoparticles, prepared using a mixture of glyceryl palmitostearate and medium-chain lipids, were labelled by mixing with 99mTc 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\footnote{153}. Radiolabelled fatty acid (131I-17-iodohopadecanoic acid) has also been used as a tracer for monitoring the fate of SLNs after a duodenal administration\footnote{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\footnote{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\footnote{155}, fluorescein isothiocyanate (FITC)\footnote{156}, and Nile red\footnote{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\footnote{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\footnote{159}. 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.\footnote{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...
Drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles

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\textsuperscript{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\textsuperscript{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.

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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 \textsuperscript{99m}Tc aqueous solution\textsuperscript{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\textsuperscript{169}. The highest activity counting was observed in the lungs as compared to other organ samples collected 4 h after inhalation\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{170}. Enhanced antitumor activity was observed when SLNs were evaluated for pulmonary co-delivery of anticancer drugs and siRNA\textsuperscript{171}. The lung tumor size was significantly reduced after inhalation of drug-loaded lipid nanoparticles with sizes around 110 nm, prepared using a mixture of glyceryl palmistearate and squalene as a lipid phase and phospholipids and Tween 80 as emulsifiers\textsuperscript{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\textsuperscript{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\textsuperscript{171}. This study confirms the prolonged retention time of solid lipid

\begin{figure}[h]
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\caption{(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.}
\end{figure}
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. 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.

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 monooleate and phosphatidylcholine as a lipid phase and poloxamer 188 as an emulsifier. 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.

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 nanocarriers in sustained topical drug delivery as well as targeting to special tissues and organs could be further explored.

**Acknowledgment**

Financial support to Anan Yaghmur for studies on development of drug nanocarriers based on cubosomes and hexosomes by the Danish Council for Independent Research | Technology and Production Sciences (references 1335-00150b and DFF- 7017-00065, Denmark) is gratefully acknowledged.

**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, Vauchier 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. *Expert 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, Waglew ska E, Bazylinski 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. *Expert Opin Drug Deliv* 2020;17:963–92.
14. Tapinos 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-Galano 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, Kenwar 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, psychochemicals 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, Sagulowicz 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, Sagulowicz 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 phytytrioil: effect of vitamin E acetate and F127 polymer on liquid crystal nanostucture. *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 monolein/oleic acid/Pluronnic 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, Antopolosky M, Airaksinen AJ, Urri T, Yaghmur A, et al. Hexosome engineering for targeting of regional lymph nodes. *Materialia* 2020;11:100705.
31. Yaghmur A, Rappol M, Jonassen ALU, Schmitt M, Larsen SW. In situ monitoring of the formation of lipidic non-lamellar liquid crystal depot formulations in synovial fluid. *J Control 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, Sturnup 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, Sturnup S, Gammelgaard B, Urri T, Moghimi SM, et al. Cisplatin encapsulation generates morphologically different multicompartment in the internal nanostructures of nonlamellar liquid-crystalline self-assemblies. *Langmuir* 2018;34:6570–81.
Drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles
111. Tran N, Bye N, Moffat BA, Wright DK, Cuddihy A, Hinton TM, et al. Dual-modality NIRF-MRI cubosomes and hexosomes: high throughput purification and in vivo biodistribution. *Mater Sci Eng C Mater Biol Appl* 2017;71:584–93.

112. Bifi S, Andolfi L, Caltagirone C, Garroco 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. Pacitaxel loaded PEGylated glycerol monooleate based nanoparticulate carriers in chemotherapy. *Biomaterials* 2012;33:7206–20.

115. Zhai J, Tan F, Luwor R, Srivinasa 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;3:1198–207.

116. Tian Y, Li JC, Zhu JX, Zhu N, Zhang HM, Liang L, et al. Folic acid-conjugated poly(ethylene oxide-co-butyl acrylate) and poly(ethylene oxide-co-polyethylene glycol) nanoparticles for oral delivery of paclitaxel: In vitro and in vivo evaluation. *Int J Pharm* 2017;520:63–72.

117. Grislin L, Couvreur P, Lenaerts V, Roland M, Deprez-Decampeneere D, Speiser P. Pharmacokinetics and distribution of a biodegradable drug-carryer. *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 nanoparticles: 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, Bo RN, Huang Y, et al. Cubosome nanoparticles potentiate immune properties of immunostimulants. *Int J Nanomed* 2016;11:5371–83.

122. Rizwan SB, McBurney WT, Young K, Hanley T, Boyd BJ, Rades T, et al. Cubosomes containing the adjuvants iniquimod 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 targeted delivery of drug loaded nanoparticles. *Int J Pharm* 2004;283:71–80.

124. Zhang QN, Yang H, Li X, Yang YQ, Wang H, et al. The effectiveness of folic acid-conjugated paclitaxel-loaded solid lipid nanoparticles in breast tumor therapy. *Chem Biol Interact* 2016;242:119–28.

125. Mu HL, Holm R. Solid lipid nanoparticles in drug delivery: characterization and design. *Expert Opin Drug Deliv* 2018;15:711–85.

126. Christophersen PC, Zhang L, Mullertz A, Nielsen HM, Yang M, Mu H. Solid lipid particles for oral delivery of peptide and protein drugs I — the digestion of triacylglycerols and monoglycerides in the gastrointestinal tract and its impact on epithelial cell uptake of nanoparticles. *Biomacromolecules* 2019;20:1789–97.

127. Wiedenmann V, Ochlike K, van der Schaaf U, Schrader K, Karstein HP. Heat stability of differently stabilized solid lipid nanoparticles in the presence of excess bulk phase protein. *Food Bioprocess Technol* 2019;14:393–402.

128. Bove N, Wu C, Mu H. Solid lipid nanoparticles for oral delivery of peptide and protein drugs II — the digestion of triacylglycerols and monoglycerides in the gastrointestinal tract and its impact on epithelial cell uptake of nanoparticles. *Biomacromolecules* 2019;20:1789–97.

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, Ochlike K, van der Schaaf U, Schrader K, Karstein HP. Heat stability of differently stabilized solid lipid nanoparticles in the presence of excess bulk phase protein. *Food Bioprocess Technol* 2019;14:393–402.

131. Maretti E, Rustichelli C, Guatieri ML, Costantino L, Siligardi C, Miselli P, et al. The impact of lipid corona on rifampicin intramacrophage transport using inhaled solid lipid nanoparticles surface-decorated with a mannoseylated surfactant. *Pharmaceuticals* 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, Hye 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. Epprecht S, Sajjadi M, Grishin A. Enhanced oral bioavailability of fexazaine by solid lipid nanoparticles: in vitro drug release and pharmacokinetics studies. *BioMed Res Int* 2014;364304.

137. Elbashy IA, Ibrahim HM, Ismael HR, Kasem AA. Enhancing bioavailability and controlling the release of gelbein from optimized solid lipid nanoparticles. *J Drug Deliv Sci Technol* 2017;38:78–89.

138. Shangguan MZ, Lu Y, Qi JP, Han J, Tian QX, Zie YC, et al. Binary lipid–based nanostructured lipid carriers for improved oral bioavailability of silmarin. *J Biomat Sci Appl* 2014;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 lutetin. *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 tacrolimus with solid lipid dispersion formulations. *AAPS PharmSciTech* 2015;16:1091–100.

143. Borkar N, Xia DN, Holm R, Gans Y, Mullertz A, Yang MS, et al. Investigating the correlation between in vitro 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 Pharmaceut Sci* 2013;43:473–80.

145. Holm R, Porsgaard T, Porter CJH, Hoy CE, Edwards GA, Mullertz A, et al. Lymphatic fatty acids in canine with pharmaceutically formulated structured TAG in rats. *Adv Drug Deliv Rev* 2018;121:71–82.
Drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles

151. Singh I, Swami R, Khan W, Sistla R. Lymphatic system: a prospective area for advanced targeting of particulate drug carriers. *Expt 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 chylomicon 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, Elgowary MM, Kamel NM, et al. Inhalable particulate drug delivery systems for lung cancer therapy: nanoparticles, microcrystals, nanocomposites and nanoaggregates. *J Control Release* 2018;269:374—92.

168. Semmler-Behrke M, Takenaka S, Fertsch S, Wein 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. Formulaition, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. *J Control Release* 2010;144:233—41.

171. Taratula O, Kuzmov A, Shab 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.