Biophysical Reviews (2023) 15:553–567
https://doi.org/10.1007/s12551-023-01089-y

REVIEW

A short review on the applicability and use of cubosomes as nanocarriers

Amanda Santos Palma[1,2] · Bruna Renata Casadei[1] · Mayra Cristina Lotierzo[3] · Raphael Dias de Castro[3] · Leandro Ramos Souza Barbosa[1,2]

Received: 18 April 2023 / Accepted: 28 June 2023 / Published online: 7 August 2023

Abstract

Cubosomes are nanostructured lipid-based particles that have gained significant attention in the field of drug delivery and nanomedicine. These unique structures consist of a three-dimensional cubic lattice formed by the self-assembly of lipid molecules. The lipids used to construct cubosomes are typically nonionic surfactants, such as monoolein, which possess both hydrophilic and hydrophobic regions, allowing them to form stable, water-dispersible nanoparticles. One of the key advantages of cubosomes is their ability to encapsulate and deliver hydrophobic as well as hydrophilic drugs. The hydrophobic regions of the lipid bilayers provide an ideal environment for incorporating lipophilic drugs, while the hydrophilic regions can encapsulate water-soluble drugs. This versatility makes cubosomes suitable for delivering a wide range of therapeutic agents, including small molecules, proteins, peptides, and nucleic acids. The unique structure of cubosomes also offers stability and controlled release benefits. The lipid bilayers provide a protective barrier, shielding the encapsulated drugs from degradation and improving their stability. Moreover, the cubic lattice arrangement enables the modulation of drug release kinetics by varying the lipid composition and surface modifications. This allows for the development of sustained or triggered drug release systems, enhancing therapeutic efficacy and reducing side effects. Furthermore, cubosomes can be easily modified with targeting ligands or surface modifications to achieve site-specific drug delivery, enhancing therapeutic selectivity and reducing off-target effects. In conclusion, cubosomes offer a versatile and promising platform for the delivery of therapeutic agents. In this manuscript, we will highlight some of these applications.

Keywords Cubosomes · Drug delivery · SAXS · Nanoparticles · DLS · Monoolein · Phytantriol · Cryo-EM · Nanotechnology

Introduction

Drug delivery systems are nanostructures widely used in pharmaceutical research and clinical contexts to increase drug efficacy. This includes the anticancer, antimicrobial, and local anesthetic, among other drugs (Ban et al., 2019; Ghosh et al., 2019; Sokullu et al., 2019; Perez et al., 2022). These systems can overcome several problems associated with traditional medications, such as low aqueous solubility, high toxicity, low bioavailability, and non-specific distribution in the organism (Kumari et al., 2023; Onugwu et al., 2023). This is related to the need for lower drug doses due to the targeting and sustained release caused by the incorporation of the drug into nanoparticles. Furthermore, it can detect sick cells and promote cell punctual healing Angelova et al. (2017). This highly selective approach can reduce costs and patient pain.

✉ Leandro Ramos Souza Barbosa
lbarbosa@if.usp.br

[1] Institute of Physics, University of São Paulo, USP, São Paulo, SP 05508-090, Brazil

[2] Brazilian Synchrotron Light Laboratory (LNLS), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP 13083-100, Brazil

[3] Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, USP, São Paulo, SP 05508-000, Brazil

Nanoparticles

Previous studies have highlighted that there are several possibilities of carriers for drug delivery systems like liposomes (Zahednezhad et al., 2019; Romanelli et al., 2019); polymeric nanoparticles (Ban et al., 2019; Kim et al., 2019; Feitosa et al., 2019), carbon nanotubes (Kaur et al., 2019); solid

lipid nanoparticles (Parveen et al., 2012; Banerjee and Pillai, 2019), cubosomes (Angelova et al., 2017; Barriga et al., 2019; Malheiros et al., 2022), exosomes (Lim and Kim, 2019), metallic nanoparticles that can be made of gold, silver, iron (Anderson et al., 2019; Shen et al., 2018), among others like silicon-based nanoparticles. Each kind of nanostructure has its advantages and disadvantages, depending on factors like the drug's chemical properties (hydrophobicity or water solubility, for instance), and the target of the organism, among several others. Among the nanoparticles, cubosomes present large advantages over other drug delivery systems, as summarized below.

- Stability: Cubosomes are highly stable from the colloidal point-of-view, such property is important for drug delivery because it increases the possibility that the drug could be delivered to the target site without degradation or loss of efficacy. This stability also allows for the development of sustained-release drug delivery systems that can release the drug over an extended period of time.
- Targeted drug delivery: Cubosomes can be modified with targeting ligants, which allows for the targeted delivery of drugs to specific cells or tissues. This targeted drug delivery can improve the therapeutic efficacy of drugs and reduce the side effects associated with systemic drug delivery.
- Improved bioavailability: Cubosomes can improve the bioavailability of drugs by increasing their solubility and stability. This improved bioavailability can result in a lower dose of the drug being required, reducing the risk of side effects.
- Applications of cubosomes in drug delivery: Cancer therapy: Cubosomes can be used for the targeted delivery of anticancer drugs to cancer cells. Targeting ligands can be attached to the surface of cubosomes to selectively deliver the drug to cancer cells while minimizing damage to healthy cells.
- Anti-inflammatory therapy: Cubosomes can be used for the delivery of anti-inflammatory drugs to inflamed tissues. The unique internal structure of cubosomes allows for sustained release of the drug, which can reduce the frequency of dosing and improve patient compliance.
- Vaccines: Cubosomes can be used for the delivery of vaccines. The cubosomal structure allows for the encapsulation of both hydrophilic and hydrophobic antigens, which can improve the efficacy of the vaccine.

Cubosomes

The term cubosome was described for the first time by Kare Larsson in 1989 (Larsson, 1989). Nevertheless, the physical description of the cubic bicontinuous crystalline liquid phase was already discussed in previous years (Mariani et al., 1988). In a few words, cubosomes are nanoparticles with cubic inner structures. They can be assembled by using some specific lipid, like monoolein or phytantriol, and a non-ionic polymer (stabilizer agent), like pluronic F-127 (Barriga et al., 2019). These lipids are capable of forming viscous liquid crystalline inverted phases (hexagonal and cubic bicontinuous), due to its molecular geometry (volume and length of polar head and amphiphilic tail) and lipid packaging (a parameter that predicts the lipid phase formed)(Chong et al., 2011).

Cubosomes are composed of water channels, highly organized and structured, in three dimensions. The most common cubic structures for cubosomes are gyroid (Ia3d), diamond (Pn3m), and primitive (Im3m) Fig. 1A (Hyde et al., 1996). These periodically arranged aqueous channels are formed as a consequence of the non-zero curvature imposed on the lipid bilayers to reduce the total energy of the system (Fig. 1B). This kind of phase is considered an Infinite Periodic Minimal Surface (IPMS), as the structure is described by an infinite array of connected zero mean curvature saddle surfaces. Because of their unique structure, these particles can be used as well for highly hydrophobic drug encapsulation (in the lipophilic interior region), as a highly hydrophilic drug (in water channels region), or amphiphilic (in water/polar head from lipids interface).

To achieve a homogeneous size distribution of the formulation and lower particle size, extrusion can be utilized to prevent agglutination, sedimentation, Ostwald ripening, and droplet coalescence. Such a procedure is quite common in the production of LUVs (Large Unilamellar Vesicles), for instance (Ong et al., 2016). The comprehensive characterization of phytantriol cubosomes subjected to stressful operations like lyophilization and extrusion was developed by Malheiros et al. (Malheiros et al., 2021). Lyophilization (or freeze-drying) of nanoparticle systems can be difficult in terms of maintaining the materials' physical, chemical, mechanical, and biological properties. Thus, the authors used a combination of biophysical tools, like SAXS (small-angle X-ray scattering), DLS (dynamic light scattering), TEM (transmission electron microscopy), and NTA (nanoparticle tracking analyzer), to completely characterize the cubosomes under lyophilization and extrusion. Intriguingly, unlike liposomes, particle size is almost unaffected by the pore size of the extrusion filter. Moreover, the inner Pn3m cubic structure of the phytantriol cubosomes was kept unaltered during extrusion. Cubosomes show remarkable malleability even when subjected to extrusion in a 50 or 30 nm pore size filter, with an average size of 185 *pm* 2 nm compared to 237 *pm* 5 nm of the reference sample in ultra-pure water and 277 *pm* 3 nm compared to 229 *pm* 1 nm in PBS buffer. Polydispersion is unaffected by this method as well. The authors concluded by saying that cubosomes are extremely pliable if stressing

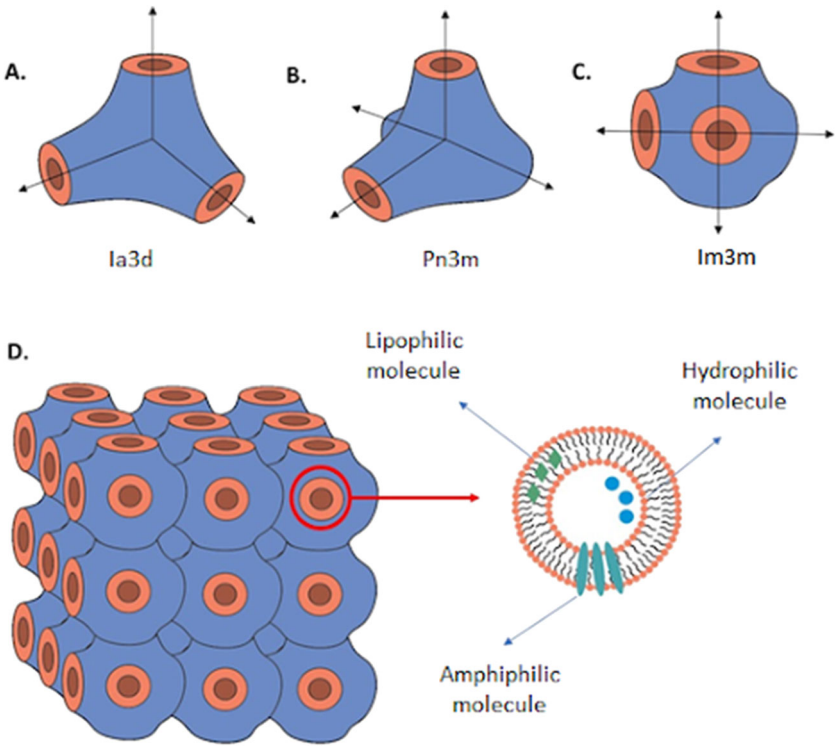

Fig. 1 Cubosomes structure. Representative scheme of cubosomes monomers in cubic bicontinuous phase **A** gyroid (Ia3D), **B** diamond (Pn3m), and **C** primitive (Im3m). **D** Cubosomes schema Pn3m demonstrating its inner structure and possible interaction with lipophilic, hydrophilic, and amphiphilic drugs

techniques can be applied to them without changing their particle size or morphology (Malheiros et al., 2021).

Cubosome composition

Mainly, there are two different lipids used to produce cubosomes, monoolein and phytantriol. Monoolein (glycerol monooleate—Fig. 2A) is a lipid, which has low aqueous solubility, very used in cubosomes production for its non-toxic, biodegradable, biocompatible, and resistant to temperature changes properties (Lai et al., 2009; Hartnett et al., 2015; Ruela et al., 2016). Its geometry favors thermodynamically the cubic structure formation because the molecule has a long alkyl chain (18 methyl groups) with an unsaturation between the 9th and 10th methylene group in the tail, and a polar head

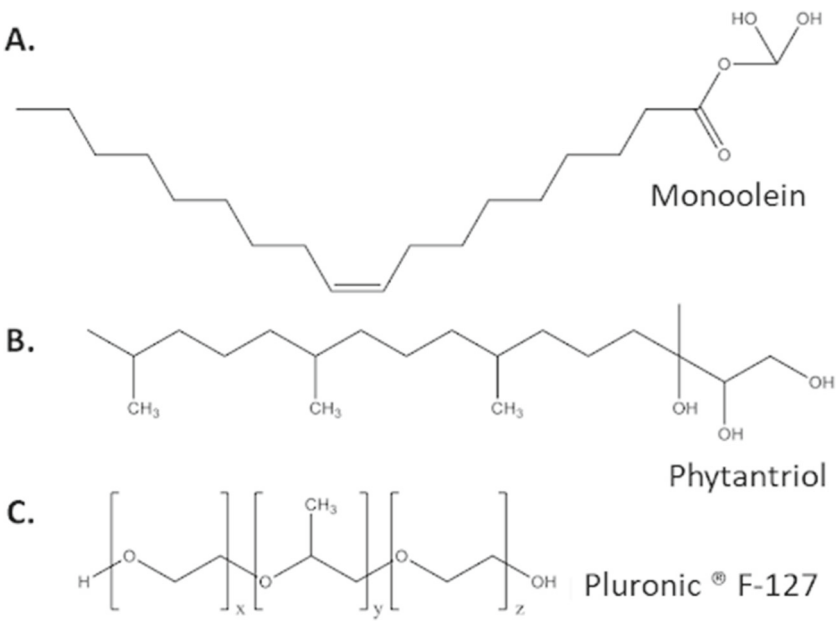

Fig. 2 Molecular structure of monoolein (**A**), phytantriol (**B**), and pluronic F-127 (**C**). **C** The letters x and z represent approximately 100 units, whereas y represents approximately 65 units

group formed by esterification of the carboxylic acid moiety and one of the hydroxyl groups from a glycerol molecule (Kulkarni et al., 2011).

It is important to point out that monoolein can form different structures in an aqueous medium, depending on the relative amount of water in the system. Figure 3A represents the monoolein phase diagram in aqueous systems. The monoolein cubic structure prevails in water excess and temperatures < 80 °C (Fig. 3A).

As an alternative to monoolein, Myverol®, a lipid mixture that contains 60% of monoolein, it is frequently used as an alternative to pure monoolein (Malheiros et al., 2021), taking a main advantage in the commercial value regarding pure monoolein. Nevertheless, the mixture of different kinds of lipids (most of them with the same length as monoolein, but with more unsaturations in the alkyl chain) may result in different crystallographic symmetries as compared to the pure monoolein.

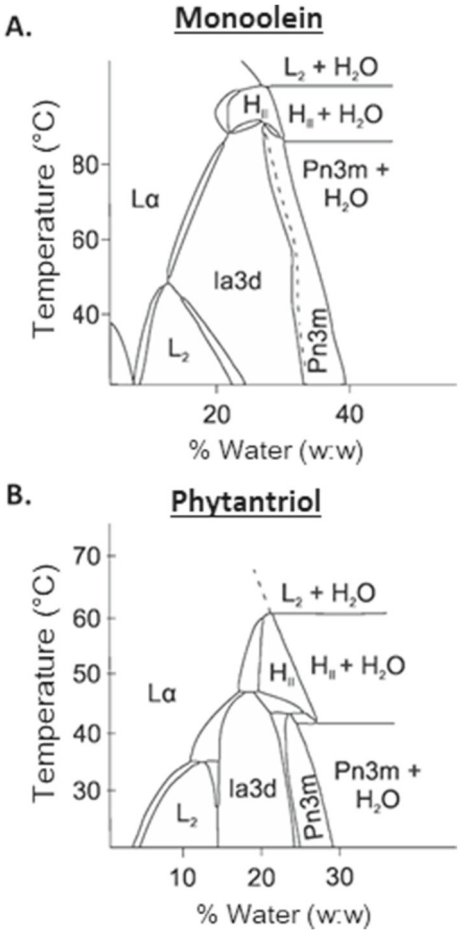

Fig. 3 Monoolein (**A**) and phytantriol (**B**) phase diagram in aqueous solution at different temperatures (Cherezov et al., year; Qiu and Caffrey, 2000; Akbar et al., 2017; Barauskas and Landh, 2003). In the Pn3m and Ia3d phases, the diagram's cubic portion is indicated for both lipids in different hydration levels. On the other side, there are the presence of some others crystallographic phases, like lamellar L_α, reverse micelles L_2, and hexagonal phase H_{II}

Phytantriol (Fig. 2B) gained a greater interest in the biomedical field, as compared to monoglycerides, due to its higher chemical stability (associated with the absence of ester group in the polar head) and also its commercial availability with 95% of purity. In contrast, monoglycerides are produced from several sources that have significantly different purity (Angelova et al., 2017). In its phase diagram (Fig. 3), it is possible to verify that the cubic structures (Pn3m and Ia3d) are predominant. However, other phases can also be found for smaller hydration (L_2, inverse, and L_α) and hexagonal (H_{II}), similarly to the monoolein phase diagram (Fig. 3B).

For colloidal stabilization, the cubosomes require the addition of a polymer, non-ionic commonly, to avoid aggregation. In this context, Pluronic® F-127, also known as poloxamer 407 (Fig. 2C), is a linear triblock polymer, non-ionic, that contains one polypropylene oxide block (nonpolar group) between two polyethylene oxide blocks (polar group). It acts like a stabilizer through absorption and incorporation of its hydrophobic blocks in the nanoparticles' surface, maintaining the internal cubic phase structure (Barriga et al., 2019).

As an alternative to Pluronic® F-127, Tween-80 polymer is being used in the cubosomes preparation (Yepuri et al., 2019; Azhari et al., 2016). Tween 80, also known as Polysorbate 80, is a non-ionic surfactant commonly used in the formulation of nanoparticles due to its unique properties such as high solubility in water, low toxicity, and excellent biocompatibility. In recent years, Tween 80 has gained attention for its potential role in improving the delivery of nanoparticles across the blood-brain barrier (BBB), a highly selective and semipermeable membrane that separates the brain from the rest of the circulatory system. Azhari et al. (Azhari et al., 2016) determined whether phytantriol-based cubosomes could be stabilized using Tween 80, thereby permitting the delivery of macromolecular medicines to the brain. Interestingly, a variety of lyotropic mesophases were produced by adding Tween 80 to mixtures of phytantriol and water during phase behavior experiments. Particularly notable features are a sizable cubic phase zone and a two-phase region with easily distributed cubosomes. Using the solvent precursor approach, cubosomes with various amounts of Tween 80 and phytantriol as the liquid crystal-forming lipid were created. Their physical characteristics were then examined by the authors (Azhari et al., 2016). The development of well-defined cubosomes with a limited size distribution and the Im3m cubic structure is demonstrated by combining DLS, Cryo-TEM, and SAXS. Collectively, the authors state that their findings show that Tween 80 may successfully maintain phytantriol cubosomes, paving the way for potential future use in BBB drug delivery.

Preparation strategies

The cubosomes can be prepared in pure water or a buffer system, with controlled pH (Negrini and Mezzenga, 2011; Ribeiro et al., 2019). The advantage of controlling pH is the possibility to develop formulations of *drug delivery* that respond more efficiently to acid or alkaline environments, which would facilitate drug delivery to target cells (Fong et al., 2016). There are, in the literature, various ways to prepare cubosomes. The main and most used are called the *top-down* method, in which the *bulk* phase is fragmented in nanoparticles, and the *bottom-up* method, in which small nanoparticles cluster together in bigger ones in dispersion (Fig. 4) (Karami and Hamidi, 2016).

Top-down In the *top down* method, the lipid and polymer are cast together and homogenized. The melt temperature varies between 40 and 70 °C, depending on the composition used. Thereafter, an aqueous medium (water or buffer solution) is added to the mixture, enabling the bulk phase (lipid/polymer mixture in water excess) to form. Subsequently, this phase is broken by an intense external energy source (sonication for example) (Spicer et al., 2001), and, at last, cubosomes dispersions are ready. Variants of this method include the use of different forms of energy, such as sonic baths, sounding tips, or homogenizers (Murgia et al., 2010; Esposito et al., 2016; Shi et al., 2017). This method is the most used in the literature for cubosomes production, although it is the method that most present polydispersion due to the energy sources (Azmi et al., 2015).

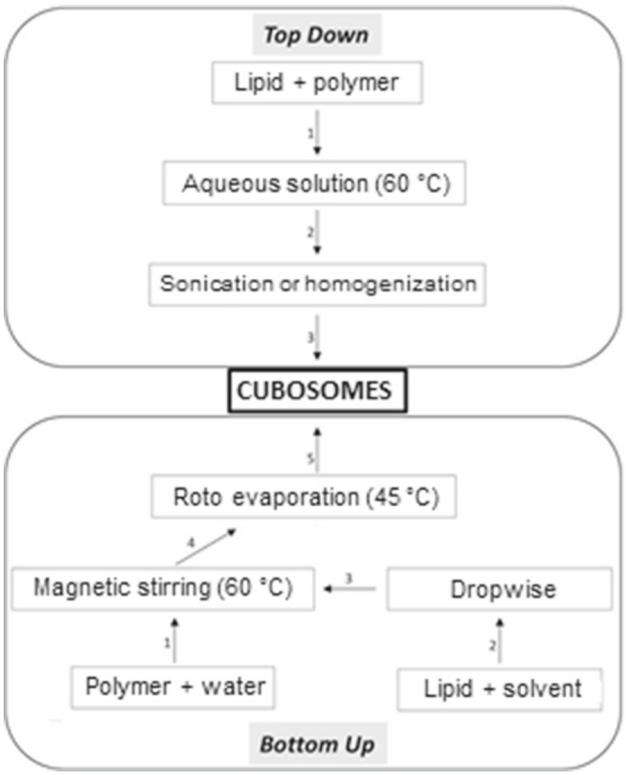

Fig. 4 Representative scheme of the principal cubosomes preparation techniques: *top down (TD)* and *bottom up (BU)*. In the TD method, the bulk phase is formed first, with lipid and polymer fusion, so it can be broken into nanoparticles by sonication or homogenization. In the BU method, two solutions are made, one lipid solubilized in a solvent and another polymer in an aqueous medium. Afterward, the lipid solution is dripped into the polymer solution where it forms the cubosomes. In the end, the use of a rotary evaporator ensures solvent removal of the nanoparticles solution

Kulkarni et al. (Kulkarni et al., 2017) used Dimodan U to prepare cubosomes with structures Im3m, Pn3m, and Ia3d loaded with aspirin (model drug) by the top-down procedure. They measured the entrapment efficiency (EE) from 84 to 96% for bulk cubic phases and 62 to 72% for cubosome dispersion. Release assays showed a more efficient release in PBS buffer than in water, showing a release percentage around 40% in physiological conditions.

Bottom up In the bottom-up method, two solutions are made: first, the lipid is solubilized in a hydrotrope (usually ethanol) solvent. Secondly, the polymer is placed in an aqueous solution (that can also be a buffer solution) at $45^{o}C$. Afterward, the solution that contains lipids is dripped into the polymer's solution, producing the nanoparticles in this stage. The resulting solution stays in the magnetic stirrer to ensure its homogeneity. At last, the cubosomes dispersion is ready, and the hydrotrope may or may not be evaporated. In this method, the use of rotary evaporators and thermal baths is quite common (Akhlaghi et al., 2016; Rizwan et al., 2007; Sherif et al., 2014). In some cases, the *bottom up* method is supplemented by sonication or homogenization to reduce the size of the nanoparticles.

Cubosome characterization techniques

There are several characterization techniques available to determine the morphology and cubosomes' inner nanostructure, from basic techniques, such as dynamic light scattering (DLS), to advanced techniques, such as cryogenic transmission electron microscopy (cryo-TEM) and small angle X-ray scattering (SAXS).

Dynamic light scattering

Dynamic light scattering (DLS) is a technique widely applied in the characterization of nanometric and colloidal systems (Hassan et al., 2015). Notably, the DLS technique has become an important tool for physical characterization, in nanosystems area used in pharmaceutical applications, for example, since, with this method, not only the size and polydispersion system under study can be accessed, but as well as the system colloidal stability itself. Part of its popularity is credited to the availability of compact equipment with a pretty good

user-equipment interface (Bhattacharjee, 2016), promoting good quality data and high reproducibility.

In a few words, this method is based on consistent light dispersion (laser) by widespread particles in a solution in order that only the Brownian motion regulates its movement (Eiser, 2014). Particles that move under such conditions have their translational diffusion coefficient (D_t) reliant on their sizes: small particles tend to have a higher diffusion coefficient, while large particles have smaller values. The correlation between the translational diffusion coefficient and the equivalent sphere size of the same D_t can be obtained through the Stokes-Einstein equation:

$$D_t = \frac{k_B T}{3\pi \eta D_H} \tag{1}$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the medium viscosity in the absence of nanoparticles. D_H is the hydrodynamic diameter of an equivalent sphere. It is worth mentioning that inside this approximation, the nanoparticles are considered spheres with a diameter (D_H) moving only by the action of the Brownian motion. This fact implies directly that there should not exist any other kind of interaction between the nanoparticles.

Cryogenic transmission electron microscopy

The cryo transmission electron microscopy (cryo-TEM) is commonly used in the study of biological materials, especially nanoparticles, such as liposomes, vesicles, and cubosomes (Burrows and Penn, 2013; Franken et al., 2017; Stewart, 2017). In the case of cubosomes, this technique is extremely valuable, once it can be seen the nanoparticles' internal morphology with rich details (Fig. 5) (Esposito et al., 2016). The advantage of using cryo-TEM is that the sample is vitrified. That means the water inside channels in the cubosome does not come out, and therefore, there is no loss of morphology. Additionally, recent detailed studies show that the water channel disposition (given the crystallographic structure) can be reconstructed by cryo tomography (Demurtas et al., 2015).

In addition to the visual information about cubosomes, the detailed micrograph analysis also reveals information about each nanoparticle's characteristic individuality (Manaia et al., 2017). Doing an analysis of fast Fourier transform (*Fast Fourier Transform*, FFT) with the images, it is possible to estimate a lattice parameter for each cubosome to complement SAXS measures (Manaia et al., 2017; Sagalowicz et al., 2007).

The technique is based on freezing the sample by dispersion immersion, followed by electron microscopy visualization. It is possible to investigate colloids in a vitrified, hydrated, and freeze state, that is really close to its native state, and reveal information about the samples' internal integrity and three-dimensional structure ((Kuntsche et al., 2011; Helvig et al., 2015)). Finally, cryo-TEM can be used to investigate morphological alterations due to drug encapsulation and preparation methodology used for cubosomes' development (Barauskas and Landh, 2003; Esposito et al., 2016; Akhlaghi et al., 2016).

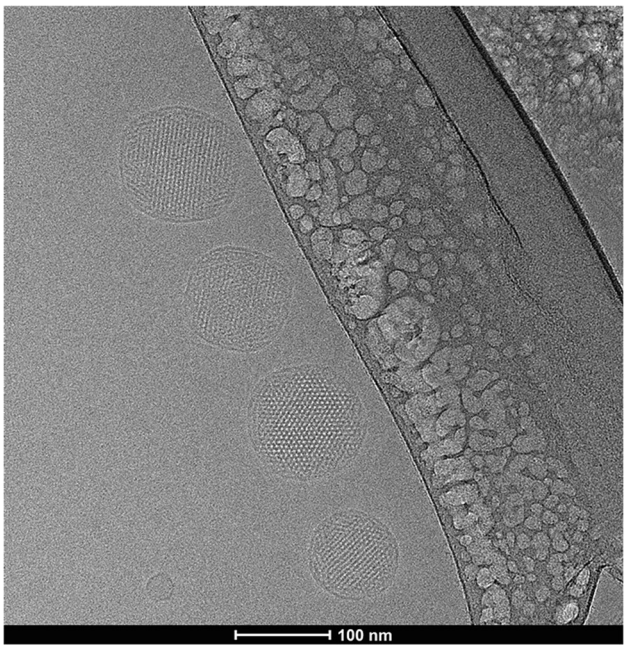

Fig. 5 Cryo-EM of phytantriol cubosomes. In the micrographs, it can be seen that nanoparticles have an "internal lattice," such a pattern would be the water channels that form cubosomes

Small-angle X-ray scattering

Small-angle scattering (SAS) is a powerful technique for studying the structure and behavior of molecules and materials at the nanoscale, like proteins, micelles, liposomes, and cubosomes, among others. It involves measuring the scattering of X-rays or neutrons from a sample and analyzing the resulting patterns to determine the size, shape, and organization of the sample's constituents. In drug delivery, SAS has become an increasingly important tool for understanding the behavior of drug molecules and delivery systems in biological environments. This is because drug delivery systems often involve complex structures and interactions between multiple components, including polymers, lipids, proteins, and drugs. One of the key advantages of SAS is its ability to provide information about the size and shape of particles and aggregates in solution, without the need for complex

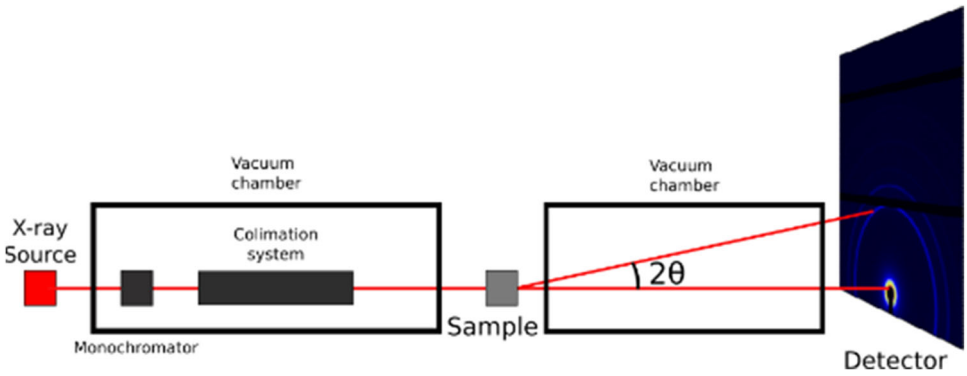

Fig. 6 Typical experimental arrangement to SAXS. Overall, conventional or synchrotron sources of X-ray are followed by very similar systems (Barbosa et al., 2013)

sample preparation or labeling. This makes it an ideal technique for studying the behavior of drug delivery systems in realistic physiological environments, such as blood or tissue fluids (Iglic et al., 2019). SAS can also provide valuable insights into the interactions between different components of drug delivery systems. For example, it can be used to study the formation of micelles or other self-assembled structures, which are often used to encapsulate drugs and improve their delivery to target tissues. SAS can also reveal information about the stability of these structures under different conditions, such as changes in pH or temperature. Concerning the case of cubosomes, SAXS can be used to calculate the unit cell parameter of the cubosomes, defining its crystallographic special group and symmetry, as follows.

The typical experimental arrangement is presented in Fig. 6. The technique's experimental principle is based on the scattering of a monochromatic X-ray light beam generated by a radiation source (conventional or synchrotron) (Barbosa et al., 2013).

In a crystalline lattice, the scattering vector **q** overlaps with the reciprocal lattice vector. If there is constructive interference on the detector, the crystalline lattice parameter can be determined. The interaction between the X-ray beam and crystallographic plane of the three-dimensional structure, related to the diffraction peak intensity in the SAXS data, determines the relation between the scattering vector value and respective Miller indices of each Bragg allowed reflection. Thus, the lattice parameter **a** can be determined by using (Marsh, 2013):

$$q = \frac{2\pi}{a}\sqrt{h^2 + k^2 + l^2} \tag{2}$$

Since each observed diffraction peak is related to the scattering vector magnitude (see Fig. 7)—each peak identified as q_1, q_2, q_3, and so on—it is possible to determine the ratio between the *ith* (q_i) and q_1, as described below, using Eq. 2:

$$\left.\begin{aligned} q_i &= \frac{2\pi}{a}\sqrt{h_i^2 + k_i^2 + l_i^2} \\ q_1 &= \frac{2\pi}{a}\sqrt{h_1^2 + k_1^2 + l_1^2} \end{aligned}\right\} \implies \frac{q_i}{q_1} = \sqrt{\frac{h_i^2 + k_i^2 + l_i^2}{h_1^2 + k_1^2 + l_1^2}} \tag{3}$$

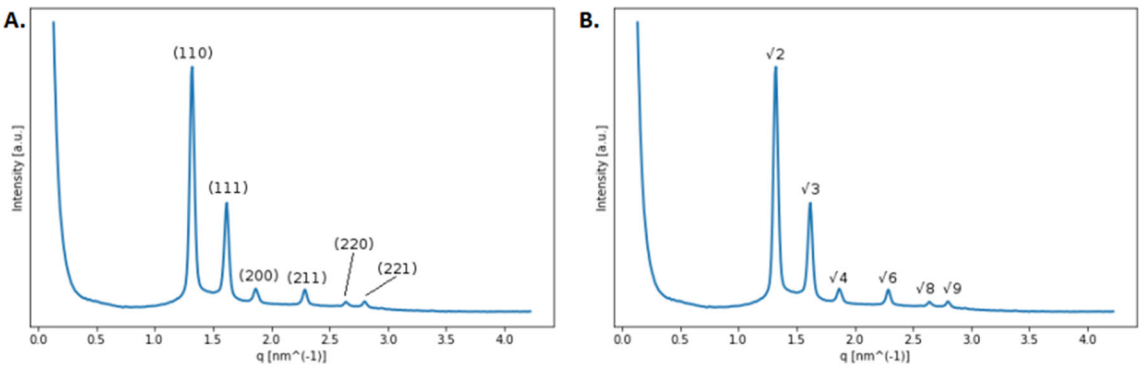

Fig. 7 Typical SAXS graphs with identified diffraction peaks. **A** Each peak is associated with Miller indices (hkl) related to Bragg reflection planes that, in turn, **B** can be associated with constants

Table 1 Values for the cubic minimal surface parameters (Mazzoni et al., 2016)

Cubic phase	A_0	χ
Pn3m	1.919	-2
Im3m	2.345	-4
Ia3d	3.091	-8

SAXS can also be used to determine other structure constants as the water channel diameter as follows:

$$\frac{d_w}{2} = r_w = a\sqrt{\frac{-\sigma}{2\pi\chi}} - l_c \tag{4}$$

where a is the lattice parameter, l_c is the alkyl length (which generally is assumed constant (Malheiros et al., 2022)), and σ and χ are topological constants. For each cubic symmetry, they assume specific values, as described in Table 1 These parameters are better described elsewhere (Malheiros et al., 2022; Marsh, 2013). Table 1 summarizes these values.

With these parameters, it is possible to calculate the cubic phase hydrophobic inner volume (ϕ_l):

$$\phi_l = 2\sigma\frac{l_c}{a} + \frac{4\pi}{3}\chi\left(\frac{l_c}{a}\right)^3 \tag{5}$$

Since the overall volume of the cubic unit cell is $V = a^3$, the volumes of the lipid and water regions in the unit cell can be simply calculated from ϕ_l and ϕ_{wat}. The number of water molecules per lipid can then be calculated using the formula (Malheiros et al., 2022):

$$N_w = \frac{v_l}{v_{water}}\frac{1-\phi_l}{\phi_l} \tag{6}$$

where v_l and v_{water} are the lipid and water molecular volumes, respectively. Lastly, one can calculate the area per lipid (A_l) (Malheiros et al., 2022):

$$A_l = \frac{2v_l}{a\phi_l}\left[\sigma + 2\pi\chi\left(\frac{l_c}{a}\right)^2\right] \tag{7}$$

Thus, the combination of SAXS and cubosomes has the potential to provide valuable insights into the structure and properties of these important nanoscale materials. Using the above equations, one can infer about the influence of external (macro)molecules on the properties of the inner cubic structure of cubosomes.

Applications and innovations

There is an interesting increase in the use of nanoparticles with biological importance that can carry bioactive molecules, especially cubosomes. The extensive surface area and the capability to incorporate lipophilic, hydrophilic, and amphiphilic compounds confer a variety of promising applications (Yaghmur and Glatter, 2009). It was stressed the importance of cubosomes as drug delivery systems; nevertheless, there are other applications to cubosomes.

For instance, cubosomes have been explored for various applications in the food and beverage industry, including the encapsulation of flavors, nutrients, and bioactive compounds, as well as improving the stability and bioavailability of sensitive ingredients (Tan et al., 2022). Cubosomes can protect encapsulated ingredients from degradation, oxidation, and other environmental factors and can also enhance their solubility, dispersibility, and sensory properties in food and beverage formulations. Cubosomes have been used in various food and beverage products, including functional foods, beverages, and nutraceuticals(Tan et al., 2022). Besides that, cubosomes have shown potential applications in medical imaging and diagnostics due to their unique optical properties. Cubosomes can encapsulate imaging agents, such as contrast agents, fluorescent dyes, and nanoparticles, and their cubic structure can provide a controlled environment for the imaging agents, leading to enhanced signal intensity and improved imaging contrast (Biffi et al., 2016). Cubosomes have been explored for various imaging modalities, including magnetic resonance imaging (MRI, (Alcaraz and Boyd, 2017)), computed tomography (CT), and optical imaging, for applications in cancer diagnosis, drug monitoring, and disease detection (Yaghmur and Mu, 2021).

Cubosomes have also been explored for agricultural and *agri-food* applications, including crop protection, nutrient delivery, and food preservation. Cubosomes can encapsulate agrochemicals, such as pesticides, herbicides, and fertilizers, and can provide a controlled release and improved efficacy of these compounds. Cubosomes can also be used for nutrient delivery in plant growth formulations to improve plant health and productivity. Additionally, cubosomes have been explored for food preservation applications, such as antimicrobial packaging, to extend the shelf-life of food products and reduce spoilage. Innovations in cubosomes nanoparticles are ongoing, and researchers are constantly exploring new applications and modifications of cubosomes to enhance their properties further and broaden their potential uses in various fields.

Some cubosomes' applications are described in the table below.

Lipid/polymer	Active principle	Action	Charecterization techniques	Ref
MO/ F127	Clopidogrel bisulfate	Myocardial infarction	TEM, DLS, DSC	(El-Laithy et al., 2019)
MO/ F127	Antimicrobial peptide LL-37	Anti-bacterial	Cryo-TEM, microbalance super-resolution laser scanning microscopy, neutron reflectivity, quartz crystal	(Boge et al., 2019)
MO/ F127	Telmisartan	Arterial hypertension	TEM, DLS, Zeta potential, DSC, Fourier Transformed Infrared Spectroscopy	(Yasser et al., 2019)
MO/ F127	Clonazepam	Antiepileptic	DLS, TEM	(El-Enin and Al-Shanbari, year)
MO/ F127	Cetrimony bromide	Effective antiseptic against bacteria and fungi	Zeta potential, SAXS, TEM	(Ni et al., 2019)
phytantriol/F127 and Myverol/F127	2,4-D 2-ethylhexyl ester, bromoxynil octanoate, P-methyl hamoxyphope	Herbicide	SAXS, DLS	(Bisset et al., 2019)
MO/F127	Donepezil	Alzheimer	DLS, Zeta potential, TEM	(Patil et al., year)
MO/F127	Sertaconazole nitrate	Antifungal	DLS, Zeta potential, TEM, DSC, Infrared	(Younes et al., 2018)
MO/F127	Clopidogrel bisulfate	Platelet aggregation inhibitor	DLS, Zeta potential, TEM	(El-Laithy et al., 2019)
MO/F127	Polysaccharide extracted from Ulva fasciata	Hyperlipidemia	DLS, DSC, TEM	(Matloub et al., 2018)
MO/F127	Cisplatin and metformin	Colorectal cancer	DLS, Zeta potential	(Saber et al., 2018)
MO/F127	Resveratrol	Antioxidant	DLS, Zeta potential, TEM	(Badie and Abbas, 2018)
MO/F127	Paclitaxel	Ovary cancer	Cryo-TEM, SAXS	(Zhai et al., 2019)
MO/F127	Timolol maleate	Glaucoma	DLS, SAXS, TEM	(Huang et al., 2017)
MO/F127	Alpha lipoic acid	Anti aging	DLS, TEM	(Sherif et al., 2014)
FIT/F127	7-Ethyl-10-hydroxycamptothecin	Anti cancer	DLS, TEM	(Ranneh et al., 2016)
FIT/F127 and MO/F127	Capsaicin	Analgesic	DLS, SAXS, TEM	(Peng et al., 2015)
FIT/F127	Vitamin E	Antioxidant	DSC, SAXS, TEM, DLS	(Dong et aL., 2006)

Cubossome functionalization and "clickability"

Alcaraz et al. (2018) investigated the ability of cubosomes functionalized with azide or dibenzocyclooctyne (DBCO) groups to engage in copper-free click chemistry with a strained cyclooctyne or azide, respectively. The authors performed this by functionalizing phospholipids with an azide or DBCO group to create cubosomes composed of phytantriol and non-ionic polymer, pluronic. Dynamic light scattering, cryo-TEM, and small-angle X-ray scattering were used to evaluate the changed cubosome size and inner structure. By treating the cubosomes with a complimentary dye and using size exclusion chromatography to distinguish between bound and unbound dye, the effectiveness of "clickability" was evaluated as well. The size, shape, and inner structure of the clickable cubosomes were only slightly altered as they interacted specifically and effectively with a click-Cy5 dye. This suggests that while taking part in copper-free click chemistry, cubosomes can maintain their distinctive internal structure. This proof-of-concept study opens the door to the application of metabolic labeling with cubosomes and copper-free click chemistry for targeted drug administration and imaging (Alcaraz et al., 2018).

Following the same clickability mentioned above, Pramanik et al. (Pramanik et al., 2022) used such a procedure to produce cubosomes containing copper acetylacetonate, a possible cancer treatment, along with hyaluronic acid (HA), a ligand for the cell surface receptor CD44. Several cancer types, including breast and colorectal cancer, have elevated levels of CD44. The interior nanostructure

of HA-tagged, copper-acetylacetonate-loaded cubosomes is based on the space group Im3m and has an average hydrodynamic diameter of about 150 nm, according to the authors. Two CD44-expressing cancer cell lines, MDA-MB-231 and HT29, which represent breast and colon cancer, respectively, efficiently took up these cubosomes, but not two CD44-negative cell lines (MCF-7 breast cancer and HEK-293 kidney cells). The effectiveness of the targeting was shown in the CD44-positive cells, where HA-tagged cubosomes dramatically increased cell death compared to untargeted cubosomes. Both were comparatively ineffective against CD44-negative cells, proving the targeting's specificity. Finally, the authors concluded that HA-tagged, copper-acetylacetonate-loaded cubosomes have enormous potential as a successful therapy for the targeted treatment of malignancies that express CD44 (Pramanik et al., 2022).

Using organic solvents during sample preparation could be a problem for encapsulating hydrophobic compounds since these small molecules may change the inner cubic structure of cubosomes. Thus, to overcome and better investigate these conditions, Lotiero et al. (Lotierzo et al., 2020) examined the structural effects of four distinct solvents (acetone, ethanol, chloroform, and octane) using SAXS and cryo-TEM. The authors observed a cubic-to-micellar phase transition in the presence of a high concentration of acetone and ethanol (1:5 solvent:PHY volumetric ratio). Interestingly, chloroform and octane exhibited differing effects on PHY-based cubosomes. Both solvents induced a cubic-to-hexagonal phase transition. These effects are ascribed to the solvent's entry into the cubosome's hydrophobic region, which increased its hydrophobic volume and caused this change. Chloroform and octane also showed a second phase transition from reversed hexagonal to inverted micellar. Moreover, the authors also followed these effects after 24 h and evidenced that some of the structural features suffered by the cubosomes were irreversible (Lotierzo et al., 2020).

Recently, Pimenta et al. (Pimenta et al., 2023) created cubosomes that are surface-functionalized with biocompatible chitosan-N-arginine and alginate. According to the authors, the pH-responsive properties of polyelectrolyte-shell (PS) cubosomes make them useful for the oral administration of drugs, for instance. The developed PScubosomes have a potent interaction with serum albumin, a protein secreted in the stomach as a result of gastric cancer. ITC measurements at 37 °C were used to define an efficient thermodynamic PScubosome-protein interaction at pH 2.0 and 7.4. Moreover, differential scanning calorimetry showed that the albumin conformation transition temperature increased significantly after being incubated with the PScubosomes. The internal liquid crystalline topology of the nanocarriers underwent significant changes, including an Im3m to Pn3m transition and a decrease in the cubic lattice parameters, according to SAXS data. In a range of temperatures, the PScubosome nanoparticle interaction with serum albumin caused internal structural changes that aided in the release of water from the cubosomal water channels. Thus, the combined findings observed by the authors demonstrated the efficient PScubosome-albumin interactions under simulated gastrointestinal pH settings and suggested promising nanocarrier properties for timed oral drug release (Pimenta et al., 2023).

Cubosomes as drug delivery systems

Akhlaghi et al. (Akhlaghi and Loh, 2017) using ultrasonication procedure, phytantriol cubosomes (Palpepcubes) containing the two palmitoyl peptides GHKcube and GQPRcube were created. DLS, cryo-TEM, and SAXS measurements were used to describe the Palpepcubes dimensions. The final one showed that, despite palmitoyl peptide concentrations as high as 5 weight percent, the bicontinuous cubic structure persisted, with an increase in cell parameters from roughly 6.5 to 7.2 nm. The interactions between the blank cubosomes and the palmitoyl peptides were investigated using isothermal titration calorimetry (ITC), which revealed an exothermic process of contact. Additionally, a continuous release lasting up to a few days was seen when the in vitro release of the palmitoyl peptides from the Palpepcubes was investigated using a dialysis method combined with the liquid chromatography-mass spectrometry (LC/MS) approach. The stability of the palmitoyl peptides and Palpepcubes stored in aqueous solutions at room temperature and at a low temperature (4 °C) was finally investigated using the LC/MS method, demonstrating that insertion into cubosomes greatly boosts peptide stability.

Untreated glaucoma, a degenerative visual neuropathy marked by elevated intraocular pressure, can even lead to blindness. Due to the variety of ocular biological barriers, which frequently result in limited or no effectiveness for medications administered through the eye, ophthalmological drug therapy is a challenge of tremendous clinical importance. On this ground, Bessone et al.(Bessone et al., 2021) proposed and characterized cubosomes as a drug delivery carrier for the glaucoma medication latanoprost. The formulations' latanoprost concentrations ranged from 0.00125 % to 0.02 wt%. Each cubosome had a similar average size of 200 nm and a low polydispersity index of 0.1; surprisingly, the authors also observed encapsulation effectiveness of nearly 90% (Bessone et al., 2021). Moreover, at least in the used concentration range, the authors did not observe any structural phase transition of the Pn3m cubic structure. Latanoprost and cubosomes interacted quickly and exothermically, according to calorimetric tests. Latanoprost release from cubosomes was gradual over time, indicating a sustained release profile, according to in vitro analyses. Finally and based on this behavior, the subconjunctival injection of CubLnp to

normotensive rabbits was used to assess the in vivo hypotensive intraocular impact, and the comparison with a commercial latanoprost formulation (0.005 percent w/v) was performed. The authors obtain really interesting results in the in vivo tests, evidencing that CubLnp could be used pharmaceutically.

One of the most prevalent malignancies impacting women worldwide is cervical cancer. Each year, there are an estimated 570,000 new cases of cervical cancer, and standard therapies can have serious adverse consequences. Thus, to overcome this issue, Victorelli et al. (Victorelli et al., 2022) created a platform for lipophilic medicines to be administered vaginally for the treatment of cervical cancer. To improve the bioavailability and local absorption of curcumin, a lipophilic medication used to treat cervical cancer, the authors developed mucoadhesive cubosomes. To enhance mucoadhesion and enable the vaginal administration of lipophilic medications, such as curcumin, they used DOTAP, a cationic lipid, in the CAM (chick embryo chorioallantoic membrane) model to evaluate their potential efficacy (Victorelli et al., 2022). The system was characterized using SAXS, cryo-TEM, and DLS, as well. Thus, the authors discovered that the curcumin delivered from using their method is retained in the vaginal mucosa using ex vivo permeation and retention studies. When curcumin was incorporated into cubosomes, in vitro cytotoxicity assays and cellular absorption revealed an enhanced cytotoxic effect of curcumin against the HeLa cell line. The CAM model assessed the in vivo antiangiogenic activity of the curcumin-loaded cubosomes (Victorelli et al., 2022).

Malheiros et al. (Malheiros et al., 2022) employed SAXS, DLS, and cryo-TEM to evaluate the influence of Miltefosine on phytantriol-based cubosomes. Miltefosine (hexadecylphosphocholine, or HePC) is an anti-leishmanial medication with a broad spectrum of antibacterial activity that was first created as a cancer treatment. Several nanotechnology-based formulations have been thought of as HePC carriers due to their severe side effects. The structural characteristics of the under-consideration nanocarriers are highly connected with the loading effectiveness and bioavailability. The structural characteristics of phytantriol (PHY) cubosomes generated under fully hydrated conditions in the presence of rising HePC concentrations were assessed in the current work. According to our research, even if the nanoparticle polydispersity index (PDI) doubled, HePC did not affect the nanoparticle average size by at least 10 mol percent HePC/PHY. On the other hand, a strong drug concentration dependence may be seen in the interior structure of the nanoparticle. The nanoparticles exhibit a bicontinuous Pn3m Cubic internal structure of up to 3.5 mol percent; the lattice parameter of this structure increases when HePC is added to the system. According to the computed structural data, mixing HePC and phytantriol increases the hydration capacity of the lipid polar surfaces. The presence of Pn3m and Im3m bicontinuous cubic phases in cubosomes indicates that the swelling of the Pn3m phase occurs for HePC concentrations higher than 7.0 mol %.

Concluding remarks

The principal application of cubosomes is as nanocarrier to drug-controlled administration, once they are composed of biodegradable lipids by simple enzymatic activity, adapted to solubilize different types of drugs in quantities higher than particles already widely used, in case, liposomes (Garg et al., 2007). In consideration, it would be a significant achievement for cubosomes to be safe and stable nanocarriers, besides improving the bioavailability of sligh soluble drugs, entail a considerable decrease in toxicity (Karami and Hamidi, 2016). It is critical to stress that a thorough comprehension of these dispersions' physicochemical characteristics and stability under various circumstances is necessary for their utilization (solutes addition, salts, temperature variation, pH). In this regard, future advancements in the study of nanoparticles, such as cubosomes, may lead to more uses in health and material science(Yaghmur and Glatter, 2009).

Future perspective for cubosome application is wide. There are many questions are still open, such as the mechanism of drug release, cubosome-cell interaction, and the importance of cubosome structure for the cell. Another issue is to establish a protocol for human treatment using cubosome formulations.

Finally, cubosomes can be functionalized with targeting ligands, such as antibodies or peptides, to achieve site-specific drug delivery. These ligands can recognize and bind to specific receptors or biomarkers present on the surface of target cells or tissues. By incorporating targeting moieties onto the surface of cubosomes, they can be guided to specific sites, improving the precision and efficacy of drug delivery. Besides this, cubosomes can be engineered to incorporate imaging agents, such as fluorescent dyes, quantum dots, or contrast agents (Zhai et al., 2022). This enables their use as multifunctional nanocarriers for both therapeutic and diagnostic purposes. By combining drug delivery capabilities with imaging functionalities, cubosomes can facilitate real-time monitoring of drug release and distribution, as well as provide non-invasive imaging of specific tissues or disease sites. Cubosomes have shown promise as vaccine delivery systems. They can effectively encapsulate antigens and adjuvants, leading to improved immune responses. Cubosomes can stabilize antigens, protect them from degradation, and provide sustained release, thus enhancing the efficacy of vaccines. Additionally, the ordered structure of cubosomes can promote antigen presentation, facilitating the activation immune system (Barriga et al., 2019).

Acknowledgements The authors thank CNPq (proc. no. 155970/2018-6), and FAPESP (2015/15822-1) for financial support. LRSB thanks CNPq for the research grant (309418/2021-6). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001

Author Contributions ASP wrote the manuscript and performed the literature research. BRC and MCL performed the literature research and helped write the manuscript. LRSB made the final corrections in the manuscript and submitted the manuscript

Funding CNPq (National Council for Scientific and Technological Development), Process: 155970/2018-6 and 309418/2021-6;
FAPESP (The São Paulo Research Foundation), Process: 2015/15822-1
CAPES (Coordination for the Improvement of Higher Education Personnel) Finance Code 001

Data Availability The data that support the findings of this study are openly available by request.

Declarations

Ethical approval Not applicable in this section

Consent to participate All co-authors had consented to participate in the manuscript.

Consent for publication We have consent of publishing.

Conflict of interest The authors declare no competing interests.

References

Akbar S, Anwar A, Ayish A, Elliott JM, Squires AM (2017) Phytantriol based smart nano-carriers for drug delivery applications. Eur J Pharm Sci 101:31–42. https://doi.org/10.1016/j.ejps.2017.01.035

Akhlaghi SP, Loh W (2017) Interactions and release of two palmitoyl peptides from phytantriol cubosomes. Eur J Pharm Biopharm 117:60–67. https://doi.org/10.1016/j.ejpb.2017.03.022

Akhlaghi SP, Ribeiro IR, Boyd BJ, Loh W (2016) Impact of preparation method and variables on the internal structure, morphology, and presence of liposomes in phytantriol-pluronic® f127 cubosomes. Colloids Surf B: Biointerfaces 145:845–853. https://doi.org/10.1016/j.colsurfb.2016.05.091

Alcaraz N, Boyd BJ (2017) Cubosomes as carriers for MRI contrast agents. Curr Med Chem 24(5):470–482. 0929-8673 ISSN

Alcaraz N, Liu Q, Hanssen E, Johnston A, Boyd BJ (2018) Clickable cubosomes for antibody-free drug targeting and imaging applications. Bioconjug Chem 29(1):149–157. https://doi.org/10.1021/acs.bioconjchem.7b00659

Anderson SD, Gwenin VV, Gwenin CD (2019) Magnetic functionalized nanoparticles for biomedical, drug delivery and imaging applications. Nanoscale Res Lett 14(1):1–16. https://doi.org/10.1186/s11671-019-3019-6

Angelova A, Garamus VM, Angelov B, Tian Z, Li Y, Zou A (2017) Advances in structural design of lipid-based nanoparticle carriers for delivery of macromolecular drugs, phytochemicals and anti-tumor agents. Adv Colloid Interface Sci 249:331–345. https://doi.org/10.1016/j.cis.2017.04.006

Azhari H, Strauss M, Hook S, Boyd BJ, Rizwan SB (2016) Stabilising cubosomes with tween 80 as a step towards targeting lipid nanocarriers to the blood-brain barrier. Eur J Pharm Biopharm 104:148–155. https://doi.org/10.1016/j.ejpb.2016.05.001

Azmi ID, Moghimi SM, Yaghmur A (2015) Cubosomes and hexosomes as versatile platforms for drug delivery. Therapeutic delivery 6(12):1347–1364. https://doi.org/10.4155/tde.15.81

Badie H, Abbas H (2018) Novel small self-assembled resveratrol-bearing cubosomes and hexosomes: preparation, charachterization, and ex vivo permeation. Drug Develop Indust Pharm 44(2):2013–2025. https://doi.org/10.1080/03639045.2018.1508220

Ban E, Kwon T-H, Kim A (2019) Delivery of therapeutic miRNA using polymer-based formulation. Drug Deliv Trans Res 9:1043–1056. https://doi.org/10.1007/s13346-019-00645-y

Banerjee S, Pillai J (2019) Solid lipid matrix mediated nanoarchitectonics for improved oral bioavailability of drugs. Expert Opinion Drug Metabolism & Toxicol 15(6):499–515. https://doi.org/10.1080/17425255.2019.1621289

Barauskas J, Landh T (2003) Phase behavior of the phytantriol/water system. Langmuir 19(23):9562–9565. https://doi.org/10.1021/la0350812

Barbosa LRS, Spinozzi F, Mariani P, Itri R (2013) Small-angle x-ray scattering applied to proteins in solution. Methods Appl Biotechnol Mater Sci, Proteins Solution Interfaces, pp 49–72. https://doi.org/10.1002/9781118523063.ch3

Barriga HM, Holme MN, Stevens MM (2019) Cubosomes: the next generation of smart lipid nanoparticles? Angewandte Chemie Int Edition 58(10):2958–2978. https://doi.org/10.1002/anie.201804067

Bessone CDV, Akhlaghi SP, Tártara LI, Quinteros DA, Loh W, Allemandi DA (2021) Latanoprost-loaded phytantriol cubosomes for the treatment of glaucoma. Eur J Pharm Sci 160:105748. https://doi.org/10.1016/j.ejps.2021.105748

Bhattacharjee S (2016) DLS and zeta potential-what they are and what they are not? J Controll Release 235:337–351. https://doi.org/10.1016/j.jconrel.2016.06.017

Biffi S, Andolfi L, Caltagirone C, Garrovo C, Falchi AM, Lippolis V, Lorenzon A, Macor P, Meli V, Monduzzi M et al (2016) Cubosomes for in vivo fluorescence lifetime imaging. Nanotechnology 28(5):055102. https://doi.org/10.1088/1361-6528/28/5/055102

Bisset NB, Webster GR, Dong YD, Boyd BJ (2019) Understanding the kinetic mixing between liquid crystalline nanoparticles and agrochemical actives. Colloids Surf B: Biointerfaces 175:324–332. https://doi.org/10.1016/j.colsurfb.2018.11.063

Boge L, Browning KL, Nordstrom R, Campana M, Damgaard LS, Seth Caous J, Hellsing M, Ringstad L, Andersson M (2019) Peptide-loaded cubosomes functioning as an antimicrobial unit against Escherichia coli. ACS Appl Mater & Interfaces 11(24):21314–21322. https://doi.org/10.1021/acsami.9b01826

Burrows ND, Penn RL (2013) Cryogenic transmission electron microscopy: aqueous suspensions of nanoscale objects. Microsc Microanal 19(6):1542–1553. https://doi.org/10.1017/S1431927613013354

Cherezov V, Clogston J, Papiz MZ, Caffrey M (2006) Room to move: crystallizing membrane proteins in swollen lipidic mesophases. J Mol Biol 357(5):1605–1618. https://doi.org/10.1016/j.jmb.2006.01.049

Chong JY, Mulet X, Waddington LJ, Boyd BJ, Drummond CJ (2011) Steric stabilisation of self-assembled cubic lyotropic liquid crystalline nanoparticles: high throughput evaluation of triblock polyethylene oxide-polypropylene oxide-polyethylene oxide copolymers. Soft Matter 7(10):4768–4777. https://doi.org/10.1039/C1SM05181D

Demurtas D, Guichard P, Martiel I, Mezzenga R, Hébert C, Sagalowicz L (2015) Direct visualization of dispersed lipid bicontinuous cubic phases by cryo-electron tomography. Nature Commun 6(1):8915. https://doi.org/10.1038/ncomms9915

Dong YD, Larson I, Hanley T, Boyd BJ (2006) Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin e acetate and F127 polymer on liquid crystal nanostructure. Langmuir 22(23):9512–9518. https://doi.org/10.1021/la061706v

Eiser E (2014) Dynamic light scattering. *Multi Length-Scale Characterisation* 233–282. https://doi.org/10.1002/9781118683972.ch5

El-Enin HA, Al-Shanbari AH (2018) Nanostructured liquid crystalline formulation as a remarkable new drug delivery system of antiepileptic drugs for treating children patients. Saudi Pharm J 26(6):790–800. https://doi.org/10.1016/j.jsps.2018.04.004

El-Laithy HM, Badawi A, Abdelmalak NS, Elsayyad NME (2019) Stabilizing excipients for engineered clopidogrel bisulfate procubosome derived in situ cubosomes for enhanced intestinal dissolution: stability and bioavailability considerations. Eur J Pharm Sci 136. https://doi.org/10.1016/j.ejps.2019.06.008

Esposito E, Mariani P, Drechsler M, Cortesi R (2016) "Structural studies of lipid-based nanosystems for drug delivery: X-ray diffraction (xrd) and cryogenic transmission electron microscopy (cryo-tem) in Handbook of nanoparticles 861–889. Springer. https://doi.org/10.1007/978-3-319-15338-4_39

Feitosa VA, de Almeida VC, Malheiros B, de Castro RD, Barbosa LRS, Cerize NNP, de Oliveira Rangel-Yagui C (2019) Polymeric micelles of pluronic F127 reduce hemolytic potential of amphiphilic drugs. Colloids Surf B: Biointerfaces 180:177–185. https://doi.org/10.1016/j.colsurfb.2019.04.045

Fong WK, Negrini R, Vallooran JJ, Mezzenga R, Boyd BJ (2016) Responsive self-assembled nanostructured lipid systems for drug delivery and diagnostics. J Colloid Interface Sci 484:320–339. https://doi.org/10.1016/j.jcis.2016.08.077

Franken LE, Boekema EJ, Stuart MC (2017) Transmission electron microscopy as a tool for the characterization of soft materials: application and interpretation. Adv Sci 4(5):1600476. https://doi.org/10.1002/advs.201600476

Garg G, Saraf S, Saraf S (2007) Cubosomes: an overview. Biol Pharm Bull 30(2):350–353. https://doi.org/10.1248/bpb.30.350

Ghosh S, Ghosh S, Sil PC (2019) Role of nanostructures in improvising oral medicine. Toxicol Rep 6:358–368. https://doi.org/10.1016/j.toxrep.2019.04.004

Hartnett TE, Ladewig K, O'Connor AJ, Hartley PG, McLean K (2015) Physicochemical and cytotoxicity analysis of glycerol monooleinbased nanoparticles. RSC Adv 5(34):26543–26549. https://doi.org/10.1039/C4RA13890B

Hassan PA, Rana S, Verma G (2015) Making sense of Brownian motion: colloid characterization by dynamic light scattering. Langmuir 31(1):3–12. https://doi.org/10.1021/la501789z

Helvig S, Azmi ID, Moghimi SM, Yaghmur A (2015) Recent advances in cryo-TEM imaging of soft lipid nanoparticles. Aims Biophys 2(2):116–130. https://doi.org/10.3934/biophy.2015.2.116

Huang J, Peng T, Li Y, Zhan Z, Zeng Y, Huang Y, Pan X, Wu CY, Wu C (2017) Ocular cubosome drug delivery system for timolol maleate: preparation, characterization, cytotoxicity, ex vivo, and in vivo evaluation. Aaps Pharmscitech 18:2919–2926. https://doi.org/10.1208/s12249-017-0763-8

Hyde S, Blum Z, Landh T, Lidin S, Ninham B, Andersson S, Larsson K (1996) The language of shape: the role of curvature in condensed matter: physics, chemistry and biology. Elsevier. ISBN 0444815384

Iglic A, Perez PL, Rappolt M (2019) Advances in biomembranes and lipid self-assembly. Academic Press. ISBN 9780081028568/ ISSN 24519634

Karami Z, Hamidi M (2016) Cubosomes: remarkable drug delivery potential. Drug Discov Today 21(5):789–801. https://doi.org/10.1016/j.drudis.2016.01.004

Kaur J, Gill GS, and Jeet K "Applications of carbon nanotubes in drug delivery: a comprehensive review," Charact Biol Nanomater Drug Deliv 113–135. https://doi.org/10.1016/B978-0-12-814031-4.00005-2

Kim KT, Lee JY, Kim DD, Yoon IS, Cho HJ (2016) Recent progress in the development of poly (lactic-co-glycolic acid)-based nanostructures for cancer imaging and therapy. Pharmaceutics 11(6):280. https://doi.org/10.3390/pharmaceutics11060280

Kulkarni CV, Wachter W, Iglesias-Salto G, Engelskirchen S, Ahualli S (2011) Monoolein: a magic lipid? Phys Chem Chem Phys 13(8):3004–3021. https://doi.org/10.1039/C0CP01539C

Kulkarni CV, Vishwapathi VK, Quarshie A, Moinuddin Z, Page J, Kendrekar P, Mashele SS (2017) Self-assembled lipid cubic phase and cubosomes for the delivery of aspirin as a model drug. Langmuir 33(38):9907–9915. https://doi.org/10.1021/acs.langmuir.7b02486

Kumari S, Goyal A, Garg M, Antonescu A, Sindhu RK (2023) Lyotropic liquid crystal system for drug delivery of astaxanthin: physical characterization and enhanced antioxidant potential. Crystals 13(1):142. https://doi.org/10.3390/cryst13010142

Kuntsche J, Horst JC, Bunjes H (2011) Cryogenic transmission electron microscopy (cryo-TEM) for studying the morphology of colloidal drug delivery systems. Int J Pharm 417(1–2):120–137. https://doi.org/10.1016/j.ijpharm.2011.02.001

Lai J, Chen J, Lu Y, Sun J, Hu F, Yin Z, Wu W (2009) Glyceryl monooleate/poloxamer 407 cubic nanoparticles as oral drug delivery systems: I. in vitro evaluation and enhanced oral bioavailability of the poorly water-soluble drug simvastatin. Aaps Pharmscitech 10:960–966. https://doi.org/10.1208/s12249-009-9292-4

Larsson K (1989) Cubic lipid-water phases: structures and biomembrane aspects. J Phys Chem 93(21):7304–7314. https://doi.org/10.1021/j100358a010

Lim W, Kim HS (2019) Exosomes as therapeutic vehicles for cancer. Tissue Eng Regen Med 16:213–223. https://doi.org/10.1007/s13770-019-00190-2

Lotierzo MC, Casadei BR, de Castro RD, Malheiros B, Barbosa LR (2020) Cubic-to-inverted micellar and the cubic-to-hexagonal-to-micellar transitions on phytantriol-based cubosomes induced by solvents. Drug Deliv Translat Res 10:1571–1583. https://doi.org/10.1007/s13346-020-00828-y

Malheiros B, de Castro RD, Lotierzo MC, Casadei BR, Barbosa LR (2021) Design and manufacturing of monodisperse and malleable phytantriol-based cubosomes for drug delivery applications. J Drug Deliv Sci Technol 61:102149. https://doi.org/10.1016/j.jddst.2020.102149

Malheiros B, de Castro RD, Lotierzo MC, Casadei BR, Mariani P, Barbosa LR (2022) Influence of hexadecylphosphocholine (miltefosine) in phytantriol-based cubosomes: a structural investigation. Colloids Surf A: Physicochem Eng Aspects 632. https://doi.org/10.1016/j.colsurfa.2021.127720

Manaia EB, Abuçafy MP, Chiari-Andréo BG, Silva BL, Junior JAO, Chiavacci LA (2017) Physicochemical characterization of drug nanocarriers. Int J Nanomed 12:4991. https://doi.org/10.2147/IJN.S133832

Mariani P, Luzzati V, Delacroix H (1988) Cubic phases of lipid-containing systems: structure analysis and biological implications. J Mol Biol 204(1):165–189. https://doi.org/10.1016/0022-2836(88)90607-9

Marsh D (2013) Handbook of lipid bilayers. CRC Press. https://doi.org/10.1201/9781420088335-38

Matloub AA, AbouSamra MM, Salama AH, Rizk MZ, Aly HF, Fouad GI (2018) Cubic liquid crystalline nanoparticles containing a polysaccharide from ulva fasciata with potent antihyperlipidaemic activity. Saudi Pharm J 26(2):224–231. https://doi.org/10.1016/j.jsps.2017.12.007

Mazzoni S, Barbosa LR, Funari SS, Itri R, Mariani P (2016) Cytochrome-c affects the monoolein polymorphism: consequences for stability and loading efficiency of drug delivery

systems. Langmuir 32(3):873–881. https://doi.org/10.1021/acs.langmuir.5b03507
Murgia S, Falchi AM, Mano M, Lampis S, Angius R, Carnerup AM, Schmidt J, Diaz G, Giacca M, Talmon Y et al (2010) Nanoparticles from lipid-based liquid crystals: emulsifier influence on morphology and cytotoxicity. J Phys Chem B 114(10):3518–3525. https://doi.org/10.1021/jp9098655
Negrini R, Mezzenga R (2011) pH-responsive lyotropic liquid crystals for controlled drug delivery. Langmuir 27(9):5296–5303. https://doi.org/10.1021/la200591u
Ni H, Xu S, Gu P, Zhang Y, Qiu T, Liu Z, Ni H, Hu Y, Liu J, Wu Y et al (2019) Optimization of preparation conditions for ctab-modified polygonatum sibiricum polysaccharide cubosomes using the response surface methodology and their effects on splenic lymphocytes. Int J Pharm 559:410–419. https://doi.org/10.1016/j.ijpharm.2019.01.060
Ong SGM, Chitneni M, Lee KS, Ming LC, Yuen KH (2016) Evaluation of extrusion technique for nanosizing liposomes. Pharmaceutics 8(4):36. https://doi.org/10.3390/pharmaceutics8040036
Onugwu AL, Nwagwu CS, Onugwu OS, Echezona AC, Agbo CP, Ihim SA, Emeh P, Nnamani PO, Attama AA, and Khutoryanskiy VV (2023)"Nanotechnology based drug delivery systems for the treatment of anterior segment eye diseases," J Controll Release 354:465–488. https://doi.org/10.1016/j.jconrel.2023.01.018
Parveen S, Misra R, Sahoo SK (2012) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging" Nanomed: Nanotechnol. Biol Med 8(2):147–166. https://doi.org/10.1201/9781315114361
Patil RP, Pawara DD, Gudewar CS, Tekade AR (2019) Nanostructured cubosomes in an in situ nasal gel system: an alternative approach for the controlled delivery of donepezil HCl to brain. J Liposome Res 29(3):264–273. https://doi.org/10.1080/08982104.2018.1552703
Peng X, Zhou Y, Han K, Qin L, Dian L, Li G, et al. (2015) "Characterization of cubosomes as a targeted and sustained transdermal delivery system for capsaicin. drug des devel ther 9:4209–18." https://doi.org/10.2147/DDDT.S86370
Perez N, Altube MJ, Barbosa LRS, Romero EL, Perez AP (2022) Thymus vulgaris essential oil+ tobramycin within nanostructured archaeolipid carriers: a new approach against Pseudomonas aeruginosa biofilms. Phytomedicine 102. https://doi.org/10.1016/j.phymed.2022.154179
Pimenta BV, Madrid RR, Mathews PD, Riske KA, Loh W, Angelov B, Angelova A, and Mertins O (2023) "Interaction of polyelectrolyte-shell cubosomes with serum albumin for triggering drug release in gastrointestinal cancer," J Mater Chem B. https://doi.org/10.1039/D2TB02670H
Pramanik A, Xu Z, Ingram N, Coletta PL, Millner PA, Tyler AI, Hughes TA (2022) Hyaluronic-acid-tagged cubosomes deliver cytotoxics specifically to cd44-positive cancer cells. Mol Pharm 19(12):4601–4611. https://doi.org/10.1021/acs.molpharmaceut.2c00439
Qiu H, Caffrey M (2000) The phase diagram of the monoolein/water system: metastability and equilibrium aspects. Biomaterials 21(3):223–234. https://doi.org/10.1016/S0142-9612(99)00126-X
Ranneh AH, Iwao Y, Noguchi S, Oka T, Itai S (2016) The use of surfactants to enhance the solubility and stability of the water-insoluble anticancer drug SN38 into liquid crystalline phase nanoparticles. Int J Pharm 515(1–2):501–505. https://doi.org/10.1016/j.ijpharm.2016.10.058
Ribeiro IR, Immich MF, Lundberg D, Poletto F, Loh W (2019) Physiological neutral pH drives a gradual lamellar-to-reverse cubic-to-reverse hexagonal phase transition in phytantriol-based nanoparticles. Colloids Surf B: Biointerfaces 177:204–210. https://doi.org/10.1016/j.colsurfb.2019.01.055
Rizwan S, Dong YD, Boyd BJ, Rades T, Hook S (2007) Characterisation of bicontinuous cubic liquid crystalline systems of phytantriol and water using cryo field emission scanning electron microscopy (cryo fesem). Micron 38(5):478–485. https://doi.org/10.1016/j.micron.2006.08.003
Romanelli MM, Costa-Silva TAD, Cunha-Junior E, Dias Ferreira D, Guerra JM, Galisteo AJ Jr, Pinto EG, Barbosa LR, Torres-Santos EC, Tempone AG (2019) Sertraline delivered in phosphatidylserine liposomes is effective in an experimental model of visceral leishmaniasis. Front Cell Infect Microbiol 9:353. https://doi.org/10.3389/fcimb.2019.00353
Ruela ALM, Carvalho FC, Pereira GR (2016) Exploring the phase behavior of monoolein/oleic acid/water systems for enhanced donezepil administration for Alzheimer disease treatment. J Pharm Sci 105(1):71–77. https://doi.org/10.1016/j.xphs.2015.10.016
Saber MM, Al-Mahallawi AM, Nassar NN, Stork B, Shouman SA (2018) Targeting colorectal cancer cell metabolism through development of cisplatin and metformin nano-cubosomes. BMC Cancer 18:1–11. https://doi.org/10.1186/s12885-018-4727-5
Sagalowicz L, Acquistapace S, Watzke HJ, Michel M (2007) Study of liquid crystal space groups using controlled tilting with cryogenic transmission electron microscopy. Langmuir 23(24):12003–12009. https://doi.org/10.1021/la701410n
Shen L, Li B, Qiao Y (2018) Fe3O4 nanoparticles in targeted drug/gene delivery systems. Materials 11(2):324. https://doi.org/10.3390/ma11020324
Sherif S, Bendas ER, Badawy S (2014) The clinical efficacy of cosmeceutical application of liquid crystalline nanostructured dispersions of alpha lipoic acid as anti-wrinkle. Eur J Pharm Biopharm 86(2):251–259. https://doi.org/10.1016/j.ejpb.2013.09.008
Shi X, Peng T, Huang Y, Mei L, Gu Y, Huang J, Han K, Li G, Hu C, Pan X et al (2017) Comparative studies on glycerol monooleate-and phytantriol-based cubosomes containing oridonin in vitro and in vivo. Pharm Develop Technol 22(3):322–329. https://doi.org/10.3109/10837450.2015.1121496
Sokullu E, Soleymani Abyaneh H, Gauthier MA (2019) Plant/bacterial virus-based drug discovery, drug delivery, and therapeutics. Pharmaceutics 11(5):211. https://doi.org/10.3390/pharmaceutics11050211
Spicer PT, Hayden KL, Lynch ML, Ofori-Boateng A, Burns JL (2001) Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). Langmuir 17(19):5748–5756. https://doi.org/10.1021/la010161w
Stewart PL (2017) Cryo-electron microscopy and cryo-electron tomography of nanoparticles. Wiley Interdiscip Rev: Nanomed Nanobiotechnol 9(2):e1417. https://doi.org/10.1002/wnan.1417
Tan C, Hosseini SF, Jafari SM (2022) Cubosomes and hexosomes as novel nanocarriers for bioactive compounds. J Agricul Food Chem 70. (5):1423–1437. https://doi.org/10.1021/acs.jafc.1c06747
Victorelli FD, Manni LS, Biffi S, Bortot B, Buzzá HH, Lutz-Bueno V, Handschin S, Calixto G, Murgia S, Chorilli M et al (2022) Potential of curcumin-loaded cubosomes for topical treatment of cervical cancer. J Colloid Interface Sci 620:419–430. https://doi.org/10.1016/j.jcis.2022.04.031
Yaghmur A, Glatter O (2009) Characterization and potential applications of nanostructured aqueous dispersions. Adv Colloid Interface Sci 147:333–342. https://doi.org/10.1016/j.cis.2008.07.007
Yaghmur A, Mu H (2021) Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles. Acta Pharm Sinica B 11(4):871–885. https://doi.org/10.1016/j.apsb.2021.02.013
Yasser M, Teaima M, El-Nabarawi M, El-Monem RA (2019) Cubosomal based oral tablet for controlled drug delivery of telmisartan: formulation, in-vitro evaluation and in-vivo comparative pharmacokinetic study in rabbits. Drug Develop Industrial Pharm 45(6):981–994. https://doi.org/10.1080/03639045.2019.1590392

Yepuri NR, Clulow AJ, Prentice RN, Gilbert EP, Hawley A, Rizwan SB, Boyd BJ, Darwish TA (2019) Deuterated phytantriol-a versatile compound for probing material distribution in liquid crystalline lipid phases using neutron scattering. J Colloid Interface Sci 534:399–407. https://doi.org/10.1016/j.jcis.2018.09.022

Younes NF, Abdel-Halim SA, Elassasy AI (2018) Corneal targeted sertaconazole nitrate loaded cubosomes: preparation, statistical optimization, in vitro characterization, ex vivo permeation and in vivo studies. Int J Pharm 553(1–2):386–397. https://doi.org/10.1016/j.ijpharm.2018.10.057

Zahednezhad F, Saadat M, Valizadeh H, Zakeri-Milani P, and Baradaran B (2019)"Liposome and immune system interplay: challenges and potentials," J Controll Release 305:194–209. https://doi.org/10.1016/j.jconrel.2019.05.030

Zhai J, Bao L, Walduck AK, Dyett BP, Cai X, Li M, Nasa Z, Drummond CJ (2022) Enhancing the photoluminescence and cellular uptake of fluorescent carbon nanodots via cubosome lipid nanocarriers. Nanoscale 14(48):17940–17954. https://doi.org/10.1039/D2NR03415H

Zhai J, Fong C, Tran N, and Drummond C (2019) "Acs nano 13:6178–6206." https://doi.org/10.1021/acsnano.8b07961