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Non-lamellar lipid nanosystems for topical application

Faculdade de Ciências da Saúde

Universidade Fernando Pessoa

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Atesto a originalidade do trabalho,

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(Mohamed Somai)

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Resumo

A presente dissertação analisa o potencial dos nanossistemas lipídicos não lamelares para a aplicação tópica de fármacos, com enfoque nas suas vantagens e aplicações na administração cutânea, ocular e vaginal. O estudo está estruturado em três seções principais: uma análise dos benefícios e desafios da administração tópica de fármacos, uma análise dos nanossistemas lipídicos não lamelares, que incluem os cubossomas, hexossomas e esponjossomas, e uma discussão dos resultados no contexto da literatura existente.

Os nanosistemas lipídicos não lamelares formam nanoestruturas únicas capazes de encapsular vários agentes terapêuticos, melhorando a solubilidade, estabilidade e biodisponibilidade dos fármacos, e oferecem a possibilidade de controlar o perfil de libertação. Estes nanossistemas têm demonstrado potencial em superar as limitações das formas farmacêuticas convencionais, tornando-os adequados para diversas aplicações farmacêuticas, cosméticas e biomédicas.

O estudo para elaborar esta dissertação baseou-se numa análise bibliográfica e discussão das principais barreiras fisiológicas à permeação de fármacos quando administrados por via tópica, nomeadamente na aplicação cutânea, ocular e vaginal. São explorados os métodos de preparação e as aplicações dos cubossomas, hexossomas e esponjossomas, com uma avaliação detalhada da sua eficácia na administração tópica.

Os principais resultados deste estudo indicam que os nanossistemas lipídicos não lamelares aumentam a penetração e a libertação de fármacos nas aplicações cutâneas, revelando-se eficazes para determinadas condições da pele, como infeções, inflamação e queimaduras. Na administração ocular, estes nanossistemas melhoram a biodisponibilidade e a penetração dos fármacos, beneficiando tratamentos para glaucoma, infeções bacterianas e inflamação. Para a administração vaginal, os cubossomas demonstram-se promissores no controlo da libertação de fármacos e na melhoria da eficácia terapêutica, particularmente para infeções e tratamentos localizados.

Apesar dos resultados promissores, permanecem desafios para a produção em grande escala, aprovação regulatória e necessidade de ensaios clínicos. Garantir a reprodutibilidade e a escalabilidade é crucial para a aplicação bem-sucedida destes nanosistemas na prática clínica. Pesquisas futuras devem focar na otimização das formulações, identificação de outros fármacos que possam beneficiar deste tipo de

veiculação e realização de ensaios clínicos de maior escala para confirmar a sua eficácia e segurança.

Em conclusão, os nanossistemas lipídicos não lamelares representam uma estratégia inovadora para a administração tópica de fármacos, oferecendo vantagens significativas sobre as abordagens tradicionais. A sua capacidade de melhorar os resultados da administração de medicamentos em várias vias sublinha o seu potencial para revolucionar as aplicações terapêuticas, necessitando de mais pesquisa e colaboração interdisciplinar para realizar todo o seu potencial clínico.

Palavras-chave: Cubossomas; Hexossomas; Esponjossomas; Administração tópica; Administração cutânea; Administração ocular; Administração vaginal

Abstract

The present dissertation analyses the potential of non-lamellar lipid nanosystems for topical application of drugs, focusing on their advantages and applications in skin, ocular, and vaginal administration. The study is structured into three main sections: an analysis of topical drug delivery benefits and challenges, an analysis of non-lamellar lipid nanosystems, which include cubosomes, hexosomes, and spongosomes, and a discussion of the findings in the context of the existing literature.

Non-lamellar lipid nanosystems form unique nanostructures capable of encapsulating various therapeutic agents, improving drug solubility, stability, and bioavailability, and offer the possibility of controlling the release profile. These nanosystems have shown potential in overcoming the limitations of conventional pharmaceutical dosage forms, making them suitable for diverse pharmaceutical, cosmetic, and biomedical applications.

The study of this dissertation was based on bibliographic analysis and discussion of the main physiological barriers to the permeation of drugs when administered topically, namely in cutaneous, ocular, and vaginal routes. The preparation methods and applications of cubosomes, hexosomes, and spongosomes are explored, with a detailed evaluation of their efficacy in topical administration.

The main results of this study indicate that non-lamellar lipid nanosystems enhance drug penetration and release in skin applications, proving effective for certain skin conditions like infections, inflammation, and burns. In ocular drug delivery, these nanosystems improve drug bioavailability and penetration, benefiting treatments for glaucoma, bacterial infections, and inflammation. For vaginal delivery, cubosomes show promise in sustaining drug release and enhancing therapeutic efficacy, particularly for infections and localized treatments.

Despite the promising results, challenges remain for large-scale production, regulatory approval, and the need for extensive clinical trials. Ensuring reproducibility and scalability is critical for the successful translation of these nanosystems into clinical practice. Future research should focus on optimizing formulations, identifying other drugs that could benefit from this nanocarriers and conducting larger-scale clinical trials to confirm their efficacy and safety.

In conclusion, non-lamellar lipid nanosystems represent a cutting-edge strategy for topical drug administration, offering significant advantages over traditional approaches. Their ability to improve drug delivery outcomes across various routes underscores their potential to revolutionize therapeutic applications, necessitating further research and interdisciplinary collaboration to realize their full clinical potential.

Keywords: Cubosomes; Hexosomes; Spongosomes; Topical administration; Skin administration; Ocular administration; Vaginal administration.

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List of abbreviations, symbols and acronyms

ζ-potential: zeta potential

2D: Two-dimensional

3D: Three-dimensional

AUC: Area Under the Curve

BJO: Brucea javanica oil

CAM: Chick embryo chorioallantoic membrane

CFA: Complete freund's adjuvant

CIS: Cubosomes *in situ* gelling sponges

Cmax: Maximum concentration

CPP: Critical packing parameter

CysA: Cyclosporin A

cryo-TEM - Cryo-transmission electronic microscopy

DEX: Dexamethasone

DGMO: Diglycerol monooleate

DL: Drug loading

DOTAP: Dioleoyl-3-trimethylammonium propane

E+D: Epidermis (excluding SC) plus dermis

EE: Entrapment efficiency

EGFR: Epidermal Growth Factor Receptor

FB: Flurbiprofen

FDA: United States Food and Drug Administration

FIS: *In situ* gelling sponges

FRT: Female Reproductive Tract

GDO: Glycerol dioleate

GMO: Glyceryl monooleate

GRAS: Generally Recognized As Safe

GTX: Gatifloxacin

IND: Indomethacin

LLC: Lyotropic Liquid Crystalline

MIC: Minimum inhibitory concentration

MRSA: Methicillin-resistant *Staphylococcus aureus*

MRT: Mean Residence Time

MT: Methotrexate

nm: Nanometer

OG: Oleyl glycerate

P407: Poloxamer 407

PDI: Polydispersity index

PG: Phytanyl glycerate

PN: Pilocarpine nitrate

PHYT: Phytantriol

PTX: Paclitaxel

PVA: Polyvinyl alcohol

SC: *Stratum corneum*

SSD: Silver sulfadiazine

SIL: Sildenafil citrate

TET: Tetrandrine

TST: Thermal stimulus time

Introduction

Nanotechnology is a multidisciplinary field that uses nanoscale materials and devices. It has the potential to revolutionize electronics, materials science, energy field, medicine and pharmaceutics, addressing significant societal challenges (Sahoo et al., 2007). Research in drug delivery system development has emphasized the critical role of drug transport mechanisms. This recognition has led to an increase in the development of more appropriate and effective drug delivery systems (Drummond & Fong, 1999).

In the last decades, nanostructures, also known as nanosystems or nanocarriers, have emerged as valuable drug delivery systems, providing innovative solutions to a wide range of challenges associated with the administration of drugs. These challenges encompass issues such as low aqueous solubility, limited permeability across physiological membranes, susceptibility to instability, and the possibility of toxicity. Additionally, it is essential to consider protecting drugs against degradation from external elements like light and oxygen, as well as internal factors such as enzymatic breakdown and pH fluctuations (Sahoo et al., 2007; Pachioni-Vasconcelos et al., 2016; Awasthi et al., 2016).

Nanosystems comprise a wide range of delivery systems that can be generally classified based on their composition into organic and inorganic nanosystems. Organic nanosystems are composed of carbon-based nanomaterials (e.g., lipids or polymers), while inorganic nanosystems do not contain carbon and are composed of inorganic elements (e.g., silicon, quantum dots, silver, and gold). Additionally, nanosystems can have a hybrid composition (e.g., lipid and polymer materials or lipid/polymer and inorganic materials). Each of these systems, developed for precise drug delivery purposes, has its own set of advantages and disadvantages.

Organic nanosystems are attractive as drug delivery carriers due to their physiological advantages, including biocompatibility, biodegradability, low toxicity, and stability (Demetzos, 2016). In addition, these types of nanosystems include versatile nanostructures with a large surface area that can improve the loading capacity of a variety of hydrophilic and lipophilic drugs and control their delivery to a target site, and, consequently, reducing side effects and facilitating precise targeting of specific organs, tissues, or cells. The most commonly used organic drug delivery nanosystems include

polymeric nanoparticles, polymeric micelles, liposomes, lipid nanoparticles, nano/microemulsions, and dendrimers (Demetzos, 2016).

Lipid-based nanosystems are among the most extensively investigated drug delivery systems as they are highly advantageous in nanomedicine. These type of nanosystems can be tailored using various resources, such as biodegradable and biocompatible lipids, surfactants, and phospholipids. Numerous advantages of lipid nanosystems have been highlighted, including biocompatibility, sustained drug release, and scalability for large-scale manufacturing (Thatipamula et al., 2011). Due to the significant toxicity concerns, pharmaceutical companies have increasingly embraced lipid-based nanosystems, particularly because the lipid components are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA). Additionally, their biodegradable nature ensures that lipid nanosystems do not accumulate in the body, boosting their perceived safety profile (Qi et al., 2017). In terms of topical application, the viscosity, mucoadhesive and permeability characteristics of lipid nanosystems make them appealing for this route of administration (Zhai et al., 2019).

The structural similarity of lipid-based nanosystems to the lipids found in biological barriers is one of their main advantages. This resemblance enables interactions between the nanosystem matrix and physiological barriers, resulting in enhanced permeability. Recently, lipid-polymer hybrid nanosystems, such as non-lamellar lyotropic liquid crystalline (LLC) mesophases (e.g., cubosomes, hexosomes and spongosomes), have emerged as a promising class of advanced drug delivery systems, combining the advantages of biomimetic lipid-based nanosystems, mentioned above, and the advantages of polymer-based nanosystems, such as their high stability in biological environments (Araújo-Silva et al., 2023; Hadinoto et al., 2013; Li & Gorfe, 2015; Lúcio et al., 2021).

Topical drug delivery systems involve administering therapeutic agents directly to the skin or mucous membranes, where the drug is applied externally and can come in a variety of dosage forms, such as creams, ointments, gels, lotions, or patches. The aim of topical application may be for the drug to act locally, or it may involve permeation through the skin layers or be absorbed through the mucous membranes to reach the bloodstream or target site for localized or systemic effects (Zhai et al. 2019).

However, the efficacy of drug delivery systems for topical application (e.g., cutaneous/skin, ocular/ophthalmic, and vaginal routes) encounters significant challenges.

Notably, the use of penetration enhancers like propylene glycol and dimethyl sulfoxide can result in adverse effects, including skin damage, irritation, and unintended systemic drug absorption (Lopes et al., 2015). Moreover, conventional topical formulations tend to promote drug accumulation on the skin surface, which can lead to a rapid absorption at the application site and potential issues with patient compliance due to the greasy or sticky texture of ointments (Rapalli et al., 2020b; Zakaria, 2022). Therefore, there is a growing interest in exploring alternative topical administration methods that offer both effectiveness and safety. The development of lipid-based nanosystems presents a promising avenue for addressing the limitations of conventional topical delivery systems.

The objective of the present dissertation is to conduct a comprehensive review of the current state of knowledge about non-lamellar lipid nanosystems for topical applications, categorizing the research according to cutaneous/skin, ocular, and vaginal administration routes. This research topic was chosen due to the limited number of publications and the lack of substantial databases exclusively dedicated to these emerging non-lamellar lipid-based nanosystems for topical application. Researchers are increasingly interested in these lipid-based nanosystems because of their unique properties and promising outcomes. Additionally, we emphasize the need to validate reported advantages through scientific evidence obtained from recent studies. This highlights the importance of conducting rigorous scientific research to confirm the benefits associated with non-lamellar lipid nanosystems

To conduct this study, we used relevant search databases in the health sciences area such as PubMed, Google Scholar, and ScienceDirect. The search terms used included "Mesophases", "Cubosomes", "Hexosomes", "Spongosomes", "Topical", “Skin OR Cutaneous”, "Ocular OR Ophthalmic" and "Vaginal". These keywords were chosen to cover a wide range of studies that are relevant in non-lamellar lipid nanosystems and their applications in topical drug delivery. The selection criteria emphasized the inclusion of both recent publications and old studies to provide a comprehensive perspective. The inclusion criteria ensured that the selected studies offered meaningful insights into the development, characterization, and application of these type of nanosystems.

The information from selected articles was manually extracted and synthesized. Data was explored, with a focus on key parameters such as drug penetration, stability,

bioavailability, and therapeutic outcomes. The analysis included a critical evaluation of the methodologies, results, and conclusions presented in the research studies.

This dissertation is structured into three sections, each focusing on key aspects of topical drug delivery and non-lamellar lipid nanosystems. The first section provides an in-depth analysis of the advantages of topical administration, such as application of dosage forms at the site of desired action, reduced systemic side effects, and enhanced patient compliance, while also discussing challenges like restricted drug absorption, variability in absorption, and potential skin sensitivity. The second section on non-lamellar lipid nanosystems examines the characteristics of cubosomes, hexosomes, and spogosomes, including structure, properties, and preparation methods, highlighting their benefits such as improved drug solubility, controlled drug release, and increased bioavailability, along with their applications in pharmaceutical, cosmetic, and biomedical fields. Lastly, the discussion section analyzes the results in the context of existing literature, comparing the findings with previously reported data, critically examining the implications, addressing discrepancies or similarities, and providing insights into the current state of knowledge in the field.

1. Topical administration

Pharmaceutical technology advancements have motivated formulation scientists to investigate alternatives to oral or parenteral modes of administration in order to deliver therapeutic agents more effectively to their intended sites. Extensive research has been conducted in this field to explore topical delivery systems, which consists of administering a formulation to the skin or mucosa such as the eyes and vagina (Singh Malik et al., 2016).

One of the significant advantages of applying drugs to topical surfaces is that it avoids hepatic first-pass metabolism, degradation caused by gastrointestinal tract pH changes and enzymes, and plasma level fluctuations that occur when a drug is administered orally. Moreover, topical administration offers several other benefits, including improved patient compliance, ease and convenience of application, a painless and noninvasive administration technique, enhanced drug bioavailability, better physiological and pharmacological responses, restricted systemic toxicity, and exposure of the drug to non-targeted tissues/sites (Singh Malik et al., 2016; Patel et al., 2011).

Developing a topical delivery system is further complicated by a variety of biological barriers and physiological conditions that prevent effective drug access. Despite ongoing research into topical drugs for treating skin conditions, resistance to permeability remains a significant obstacle in the development of delivery systems (Singh Malik et al., 2016). To address these challenges, nanosystems, such as non-lamellar lipid nanosystems, offer promising advantages. These systems can enhance the delivery and efficacy of topically applied drugs by overcoming permeability barriers, thus optimizing therapeutic outcomes and expanding the potential of topical administration.

The following subtopics discuss the three main physiological barriers to drug permeation when administered topically, taking into account the three routes of topical administration that have been considered in this dissertation, namely skin, ocular and vaginal.

1.1. Skin barrier

The skin is a complex structure composed of distinct layers: the *stratum corneum* (SC), also known as the nonviable epidermis, the underlying viable epidermis layers (including *stratum* basal, *stratum* spinosum, *stratum* granulosum, *stratum* lucidum), the dermis, and the hypodermis (subcutaneous tissues). Additionally, the skin has various appendages

such as hair follicles, pilosebaceous units, apocrine and eccrine sweat glands, and nails. While the skin's extensive surface area may suggest that it would be an ideal route for drug administration, it presents an essential permeation challenge, owing to its outermost layer, the SC, which acts as a strong barrier toward drugs permeation. SC is a structured lipid-rich region that serves as a barrier to control the movement of water, oxygen, and chemicals into and out of the skin (Baroni et al., 2012; Menon et al., 2002).

Therefore, it becomes imperative to delve deeper into the structure of this layer. The different layers and appendages of the skin play crucial roles and serve as target areas for various functions. Despite the skin being an excellent local site for drug administration, it restricts the number of drugs that can penetrate its outermost layer in sufficient quantities to achieve therapeutic concentrations in its various layers or in the bloodstream when employing a transdermal delivery system (Benson et al., 2019; Proksch et al., 2008).

When topically products are used, their intended targets can encompass various skin layers, such as the epidermis, dermis, and hypodermis, as well as skin appendages (including hair follicles, sebaceous glands, sweat glands, and nails), and underlying tissues. (Benson et al., 2019).

There are three main pathways through which substances can permeate the skin: intracellular (transcellular), intercellular, and follicular pathways (Figure 1). The intracellular pathway involves the passage through the keratinocytes of the SC, requiring substances to cross multiple cell membranes and the cytoplasm. This route is generally less utilized by nanosystems due to the significant barriers presented by cell membranes and cytoplasm. (Palmer & DeLouise, 2016; Bolzinger et al., 2012).

The intercellular pathway involves navigation through the lipid matrix that surrounds the corneocytes. Substances move between the cells rather than through them, interacting with the lipid bilayers. This pathway is highly relevant for lipid-based nanosystems like cubosomes, spongosomes, and hexosomes, which can interact with the intercellular lipids and enhance the permeation of encapsulated drugs. The intercellular route follows a more complex path between epidermal cells. The specific pathway is likely influenced by the nanoparticle's size, charge, shape, and composition. (Prausnitz & Langer, 2008; Palmer & DeLouise, 2016)

The follicular pathway bypasses the SC by penetrating through hair follicles and sebaceous glands, providing direct access to deeper skin layers. This pathway can be

significant for nanosystems because hair follicles can serve as reservoirs for sustained release of encapsulated compounds (Fang et al., 2014).

Most topical products target the viable epidermis at specific sites including nerves, keratinocytes, melanocytes, Langerhans cells, and hair follicles, as depicted in Figure 1. On the other hand, transdermal products are designed to cross the skin's barriers (such as SC and other cellular and molecular barriers, like the antimicrobial barrier, Langerhans cells in the epidermal layer, enzyme systems) and reach the dermis, which is rich in blood vessels, allowing the drug to enter the systemic circulation (Ghasemiyeh & Mohammadi-Samani, 2020).

The physicochemical properties of drugs and their delivery systems (e.g., nanosystems), such as size, charge, fluidity, hydrophilicity/lipophilicity, have an important impact on skin permeation pathways (Ghasemiyeh & Mohammadi-Samani, 2020).

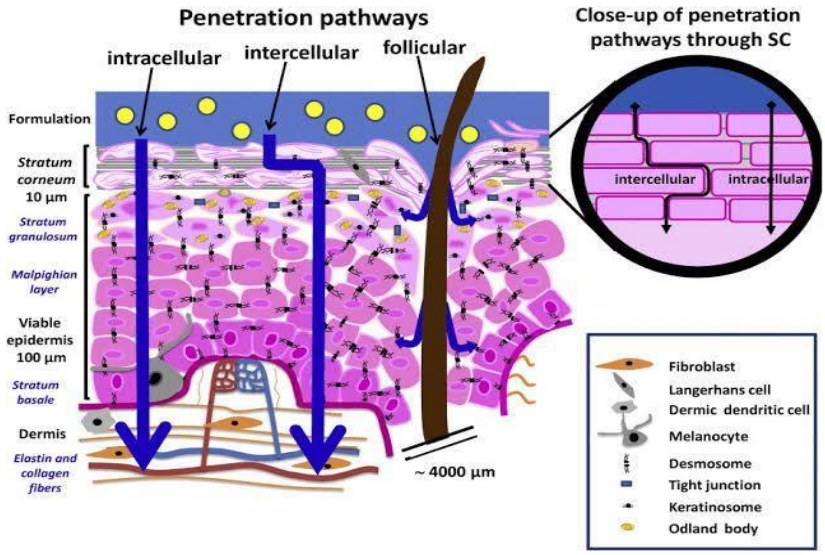

Figure 1: Schematic representation of skin penetration pathways: transcellular, intercellular, and follicular. The upper right inset is a close-up of the *stratum corneum* (SC), highlighting the transcellular and tortuous intercellular pathways.

Reprinted from Bolzinger et al. (2012). Current Opinion in Colloid & Interface Science, 17, Bolzinger et al., Penetration of drugs through skin, a complex rate-controlling membrane, pp. 156-165, Copyright (2012), with permission from Elsevier.

1.2. Ocular barrier

Addressing the challenge of delivering drugs to the eye presents a significant undertaking for ophthalmologists and pharmaceutical technologists. Topical administration in the form of eye drops is preferred for treating anterior segment diseases because it is more convenient and allows drugs to be administered locally. However, this type of

administration is hindered by issues such as limited bioavailability caused by anatomical and physiological barriers, as well as adverse ocular effects. To overcome these limitations, emerging fields like nanotechnology are pivotal in developing innovative approaches (Gan et al., 2010). These strategies are based essentially on lipid-based nanosystems (e.g., solid lipid nanoparticles) that are able to effectively cross the physiological barriers and intricate anatomical structures of the human eye (Patel et al., 2014; Luschmann & Herrmann., 2015).

The human eye is anatomically divided into two segments: anterior and posterior (Figure 2). The anterior segment encompasses several structures. The tear film, composed of lipid, aqueous, and mucin layers, has a thickness of approximately 3 μm. The cornea, a transparent dome-shaped barrier, separates the anterior chamber from the external environment (Delmonte & Kim, 2011).

Additionally, the conjunctiva, a fibrous membrane that covers the eyelids and extends over one-third of the eyeball, has a surface area 16 times larger than the cornea (Knop & Knop. 2005). Both the anterior and posterior segments contain aqueous humor, a fluid akin to blood plasma but with a lower protein content. The iris, a pigmented structure, regulates pupil diameter, while the ciliary body produces aqueous humor and houses muscles that alter lens shape. The lens itself, transparent and crucial for focusing light onto the retina, completes the components of the anterior segment. The posterior segment comprises the sclera, a dense, fibrous connective tissue that maintains eye integrity, alongside the choroid, a vascular layer that supplies nutrients to the outer retina (Nickla & Wallmann, 2010). The retina, a vascularized and innervated area, contains photoreceptor cells that convert light into neural signals which are transmitted to the brain via the optic nerve. Bruch’s membrane, a thin, multi-layered structure between the retina and choroid, supports retinal function. The vitreous humor, a gel-like substance in the eyeball's central cavity composed of collagen and hyaluronic acid, provides additional structural support. Finally, the optic nerve transmits visual information from the retina to the brain, supported by retinal blood vessels that supply the retina with essential nutrients (Forrester et al., 2016).

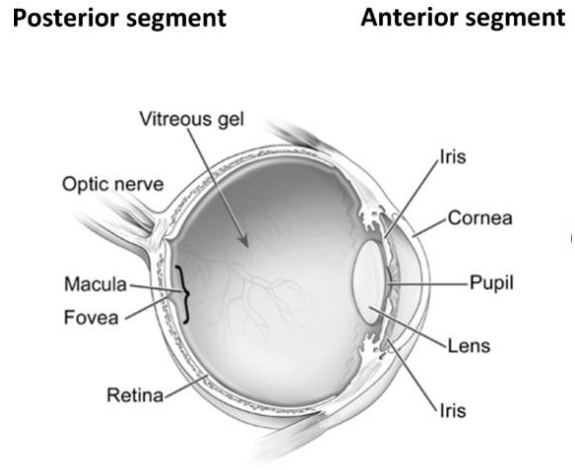

Figure 2: The cross-section of a human eye with major anterior and posterior segment parts.

Reprinted from Mahesh Kumar & Gunasundari (2018). Journal of Medical Systems, 42(7), Mahesh Kumar & Gunasundari, Computer-aided diagnosis of anterior segment eye abnormalities using visible wavelength image analysis based machine learning, p. 128, Copyright (2018).

The ocular barrier is composed of three distinct layers: the epithelium, endothelium, and inner stroma, which act as primary obstacles to drug absorption via ocular topical administration. The corneal epithelium, which faces the tear film and contains lipophilic cellular layers, prevents ion transport. Furthermore, the tight junctions within the corneal epithelium act as a selective barrier, impeding the passage of small molecules and preventing the diffusion of macromolecules through the intercellular route. The stroma, located beneath the epithelium, is predominantly hydrophilic and accounts for 90% of the corneal structure. The corneal endothelium is responsible for maintaining optimum corneal hydration. Consequently, drugs with higher lipophilicity will encounter greater resistance when attempting to cross the stroma. The physiological and anatomical limitations of the eye result in minimal drug absorption, typically around 1% or even less of the applied dose (Hirlekar et al., 2010).

Considering the several ocular obstacles (e.g., precorneal, corneal, and blood-corneal barriers), the challenge in pharmaceutical system design is to achieve the optimal drug concentration at the targeted ophthalmic sites for an appropriate drug residence time, ensuring an adequate permeation and high therapeutic effectiveness in ocular administration. Inadequate drug absorption from ocular formulations is primarily caused by factors related to pre-corneal loss, encompassing issues like solution drainage, lacrimation, tear dynamics, tear dilution, tear turnover, conjunctival absorption, brief

residence time in the cul-de-sac and the corneal epithelial membrane's limited permeability. The ocular administration can result in significant drug loss into the systemic circulation. These features pose considerable obstacles in delivering drugs to the anterior segment through topical administration (Hirlekar et al., 2010; Hughes et al., 2008).

Nanosystems represent a promising approach to address the challenges inherent in ocular drug delivery for treating anterior segment diseases. Nanosystems can improve drug ocular permeability, prolong residence time, and increase efficacy on the eye's surface. The ability of nanosystems to entrap both hydrophilic and lipophilic drugs improve drug stability, protects drugs from enzymatic degradation, and allows for sustained delivery, resulting in high bioavailability and prolonged therapeutic effect (Ahmed et al., 2023). Nanosystems design allows for precise control over drug release kinetics, with release profiles tailored to the therapeutic needs of different ocular diseases. This capability not only enhances treatment efficacy but also improves patient compliance by reducing the frequency of administrations. As a result, nanotechnology-driven approaches hold significant promise to revolutionize the management of anterior segment ocular diseases, offering safer and more effective treatment options (Bachu et al., 2018; Patel et al., 2014).

1.3. Vaginal barrier

The vaginal route of administration has developed as an appealing alternative for drug delivery because to its anatomy and physiology (Figure 3), which may produce both local and systemic effects. The vaginal route offers various benefits for drug administration compared to alternative mucosal routes, including higher absorption surface, a substantial network of lymphatic and vascular channels, avoidance of the stomach´s acidic pH, hepatic first-pass metabolic processing, and the uterine pass effect, which allows the drug to enter the uterus (Cicinelli et al., 2000; Campaña-Seoane et al., 2014). Considering the diseases and conditions of the vagina, they may be treated locally via the vaginal route, giving a high concentration of drug at the targeted site and potentially reducing any harmful effects outside of this region (Cook & Brown, 2018). The extensive surface area and abundant blood supply in the vaginal region make it a promising option for systemic drug delivery. Furthermore, it facilitates prolonged contact between drug delivery systems and the vaginal mucosa, which is easier to establish than at absorption sites like the rectum or intestinal mucosa (de Araújo Pereira & Bruschi, 2012).

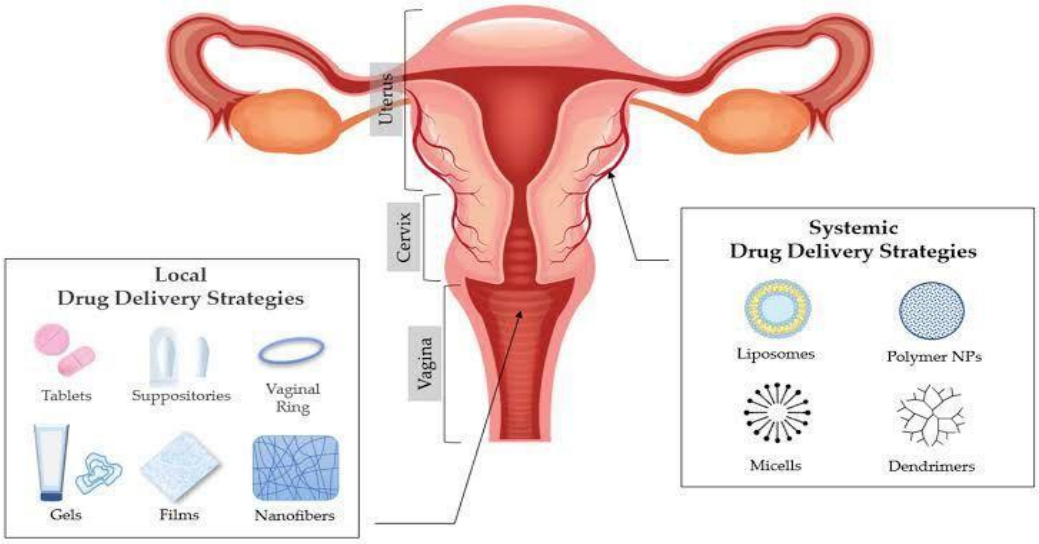

Figure 3: Anatomy of the vagina and local and systemic drug delivery strategies.

Reprinted from Dedeloudi et al., 2022 (open access paper).

The vaginal wall is composed of three layers: the epithelial layer, the muscular coat, and the tunica adventitia. The remarkable elasticity of the vagina can be attributed to the smooth elastic fibers within the muscular coat. The thickness of the non-cornified, stratified squamous tissue known as the vaginal epithelium changes with age. It increases under hormonal influence, reaching its maximum thickness during the proliferative stage, which aligns with the highest glycogen content occurring during ovulation (de Araújo Pereira & Bruschi, 2012).

After administration, the drug must be absorbed and disseminated throughout the vaginal epithelium. Transcellular transport and receptor-mediated transcytosis are the primary permeation pathways through the vaginal epithelium. The physiological factors in the vagina (e.g. vaginal epithelial thickness, cyclic changes, vaginal secretions, mucus and enzymatic activity, pH) and the physicochemical properties of drugs and their carriers (e.g. size, charge) have a substantial impact on vaginal uptake (Deshkar & Mahore, 2022).

Female reproductive tract (FRT)-related diseases affect over 10 million people annually, causing distress with symptoms like itching, burning, pain, and dyspareunia (Chindamo et al., 2021). Several studies have indicated that vaginal drug delivery is underused; therefore, great strides have lately been made to improve upon this effective route of administration (Chindamo et al., 2021). The majority of vaginal disorders originate in the uterus. According to recent statistics, 50% to 60% of females have vaginal disorders

(Patel et al., 2023). The treatment of vaginal conditions might be more effective if drugs are administered locally and under close medical supervision to avoid systemic side effects. Conventional delivery systems, which have been used for an extended period, are commonly represented by the use of traditional mucoadhesive polymers in the treatment of vaginal diseases. However, these systems have multifaceted disadvantages. Mucoadhesive polymers such as polyacrylates, chitosan, cellulose derivatives, or polysaccharides often have limited drug loading capacity, hindering their ability to deliver therapeutic amounts of drugs. Consequently, this limitation results in poor bioavailability and a shorter residence time in the vaginal mucosa. Additionally, some of these polymers may cause irritation or allergic reactions in sensitive individuals. Formulating with these polymers can be complex and may require specific expertise, complicating the manufacturing process. Moreover, the mucoadhesive properties of these polymers may vary, leading to inconsistent drug delivery and efficacy. These challenges highlight the need for alternative delivery systems to improve treatment outcomes. Nanosystems have emerged as a viable solution due to their unique advantages such as sustained delivery, increased bioavailability, efficient permeation through the vaginal epithelium, systemic absorption and higher efficacy (Patel et al., 2023).

To summarize, while the various routes of topical administration provide effective and localized drug delivery, minimizing systemic side effects and enhancing patient compliance, they also have drawbacks, such as potential skin irritation and limited penetration depth. In response to these challenges, the non-lamellar lipid nanosystems have emerged as promising drug carriers. This advanced drug delivery system utilizes unique lipid structures beyond traditional bilayers, such as cubic, hexagonal and sponge phases, to improve drug stability, targeting, and release profiles (Rizwan et al., 2013, Alves & Mano, 2015). Exploring this innovative delivery nanosystem will provide insights into how to overcome the limitations of conventional topical administration. Building on this understanding, the non-lamellar lipid nanosystem is the next topic to explore.

2. Non-lamellar lipid nanosystems: Cubosomes, Hexosomes and Spongosomes

The convergence of LLC and nanotechnology has given rise to non-lamellar lipid nanosystems, which have been explored in multiple instances for drug delivery purposes (Lancelot et al., 2014).

Lyotropic and thermotropic liquid crystals are primarily composed of organic molecules. The 2D and 3D LLC nanosystems consist of amphiphilic lipid building blocks, which serve as precursors to the bulk forms of the cubic, hexagonal, or sponge phases (Araújo-Silva et al., 2023). Thermotropic liquid crystals undergo phase transitions when the temperature changes, while lyotropic liquid crystals phase transitions depend on both temperature and concentration. LLC compounds possess the unique ability to dissolve both water-soluble and oil-soluble compounds. At low concentrations, the fundamental building block is the micelle, which is formed when polar groups within a cluster of molecules align themselves toward the water. This micellar phase is referred to as the liquid isotropic phase (Rapalli et al., 2020a; Chavda et al., 2023).

The remarkable ability of amphiphilic molecules to self-assemble into organized structures is a fundamental feature observed in various biological systems, such as the cellular membrane, endoplasmic reticulum, Golgi apparatus, and the intricately folded mitochondrial membrane. This captivating phenomenon serves as a key inspiration for the development of innovative biomimetic materials, such as LLC lipid nanosystems (Zatloukalova et al., 2023).

The formation of LLC lipid nanosystems occurs when amphiphilic lipids self-assemble into LLC mesophases in the presence of water. The hydrophobic chains separate to minimize the system's free energy and reduce oil/water contact, while the polar headgroup, which preferentially hydrated at the water interface, exemplifies the hydrophobic effect. This effect crucially facilitates the separation of hydrocarbon degradation within these formulations, promoting the self-assembly of amphiphilic lipids into LLC mesophases characterized by long-range periodic order (Zhai et al., 2019).

The self-assembly process of amphiphilic lipids produces a variety of polymorphic phases, primarily lamellar and non-lamellar structures, characterized by discrete or continuous aggregates that arrange themselves spatially in 1D, 2D, or 3D periodic patterns (Hyde, 2001b; Fong et al., 2012). Furthermore, they exhibit structures with

changing membrane stress that are favorable for improving penetration and cell internalization. Due to their inherent bicontinuous mesophases, 2D and 3D LLC nanosystems can create pores within endosomal membranes, facilitating the effective release and delivery of their cargo into the cell cytoplasm (Esposito et al., 2014; Braeckmans & Oliveira, 2014; Leal & Kim, 2015).

LLC non-lamellar nanosystems represent an emerging advanced drug delivery system, similar to liposomes (i.e. a lamellar nanosystem). However, non-lamellar lipid nanosystems exhibit complex two- and three-dimensional non-lamellar nanostructures referred to as internally self-assembled "somes" (ISAsomes) (Zhai et al., 2019). Particularly, cubosomes and hexosomes, known for their internal inverse bicontinuous cubic and hexagonal mesophases, along with spongosomes, displaying an intermediate structure, have been among the extensively studied non-lamellar lipid nanosystems (Figure 4) (Zatloukalova et al., 2023).

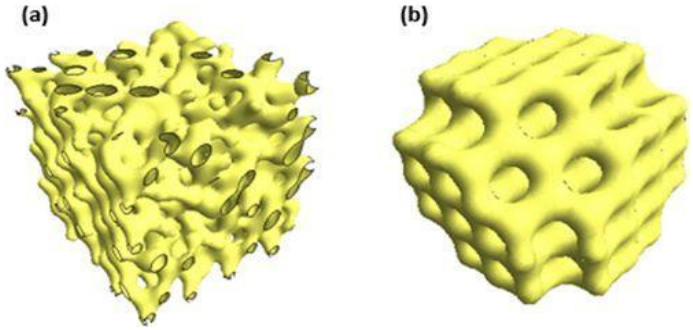

Figure 4: Schematic representation of the supramolecular organizations of spongosomes (a) and cubosomes (b).
Reprinted from Rakotoarisoa et al. 2019 (open access paper).

When compared to lamellar planar nanostructures (e.g., liposomes), highly curved non-lamellar lipid nanosystems have special features, including a greater surface area to volume ratio. This characteristic is especially useful for loading hydrophilic, hydrophobic, and/or amphiphilic therapeutic agents, whether small (e.g., drugs) or large molecules like biomacromolecules (Barriga et al., 2019). Lyotropic systems possess the notable characteristic of maintaining thermodynamically stable even in the presence of excess solvent. However, the specific types of LLC nanosystems that form is influenced by several factors, including temperature, the proportions of lipid and water, additives such as salts, pH, and geometric packing constraints of the lipids (Hyde, 2001b; Fong et al., 2012).

Among the commonly studied amphiphilic lipid building blocks to prepare LLC non-lamellar nanosystems are monoolein, often referred to as glyceryl monooleate (GMO), and phytantriol (PHYT) (Figure 5). To stabilize thermodynamically, the non-lamellar lipid nanosystems are coated with different possible stabilizers: amphiphilic block copolymers, like Pluronic® derivatives; lipids copolymer, such as Polysorbate 80; and alternative steric stabilizers (e.g., lamellar lipids, bile, proteins) (Zhai et al., 2019).

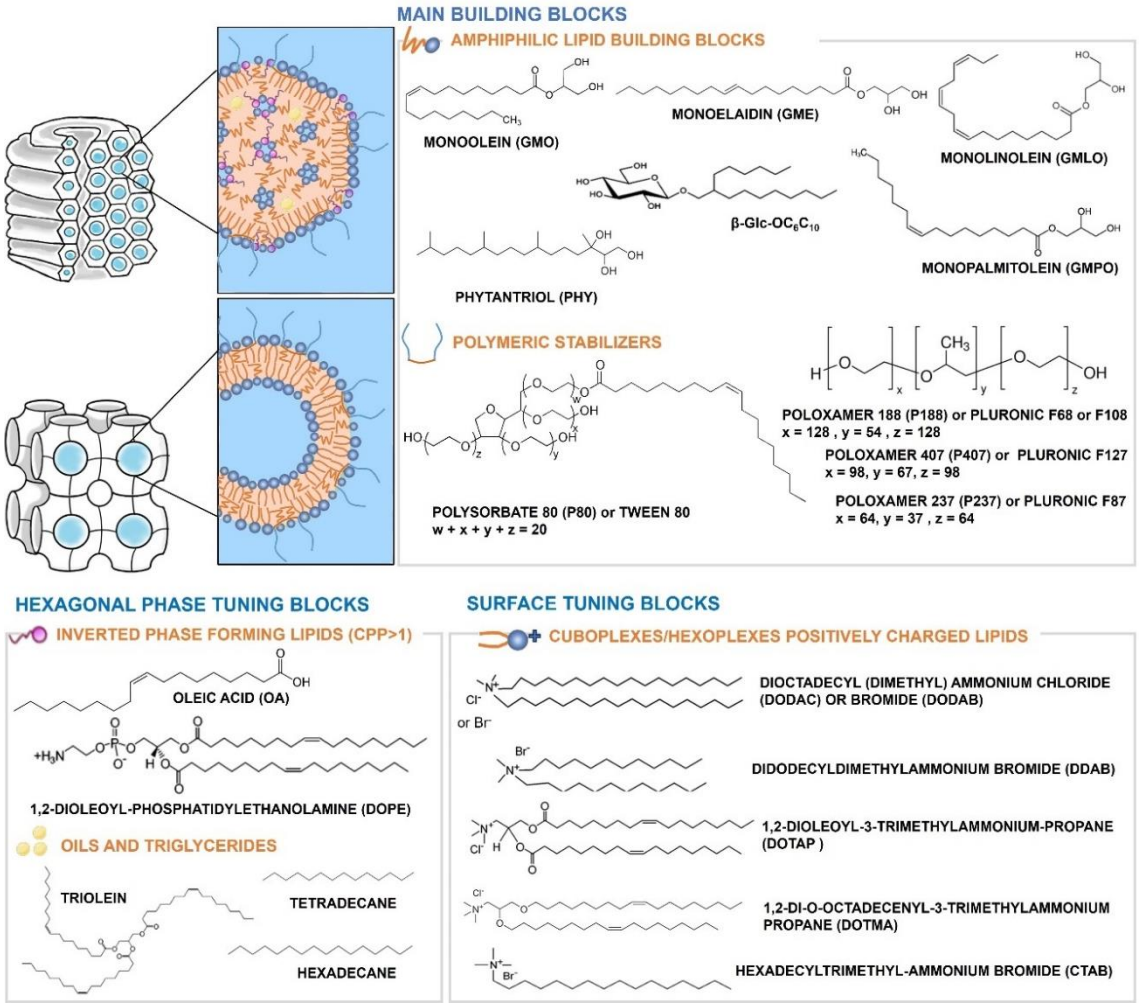

Figure 5: Molecular structures of the amphiphilic lipid building blocks and polymeric stabilizers used to produce 2D and 3D lyotropic liquid crystalline nanosystems

Reprinted from Araújo-Silva et al., 2023. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1878, Araújo-Silva et al., Lyotropic liquid crystalline 2D and 3D mesophases: Advanced materials for multifunctional anticancer nanosystems, p. 189011, Copyright (2023), with permission from Elsevier.

Aside from the hydrophobic effect that drives self-assembly, the critical packing parameter (CPP), defined as a theoretical framework for determining the type of aggregation formed by surfactants, is a useful metric for assessing aggregation topology. In the absence of interaggregate interactions, inverse or Type II non-lamellar phases favor

a CPP > 1, lamellar phases maintain a CPP = 1, and normal or Type I non-lamellar phases exhibit a CPP < 1 (Figure 6) (Araújo-Silva et al., 2023; Israelachvili, 2011).

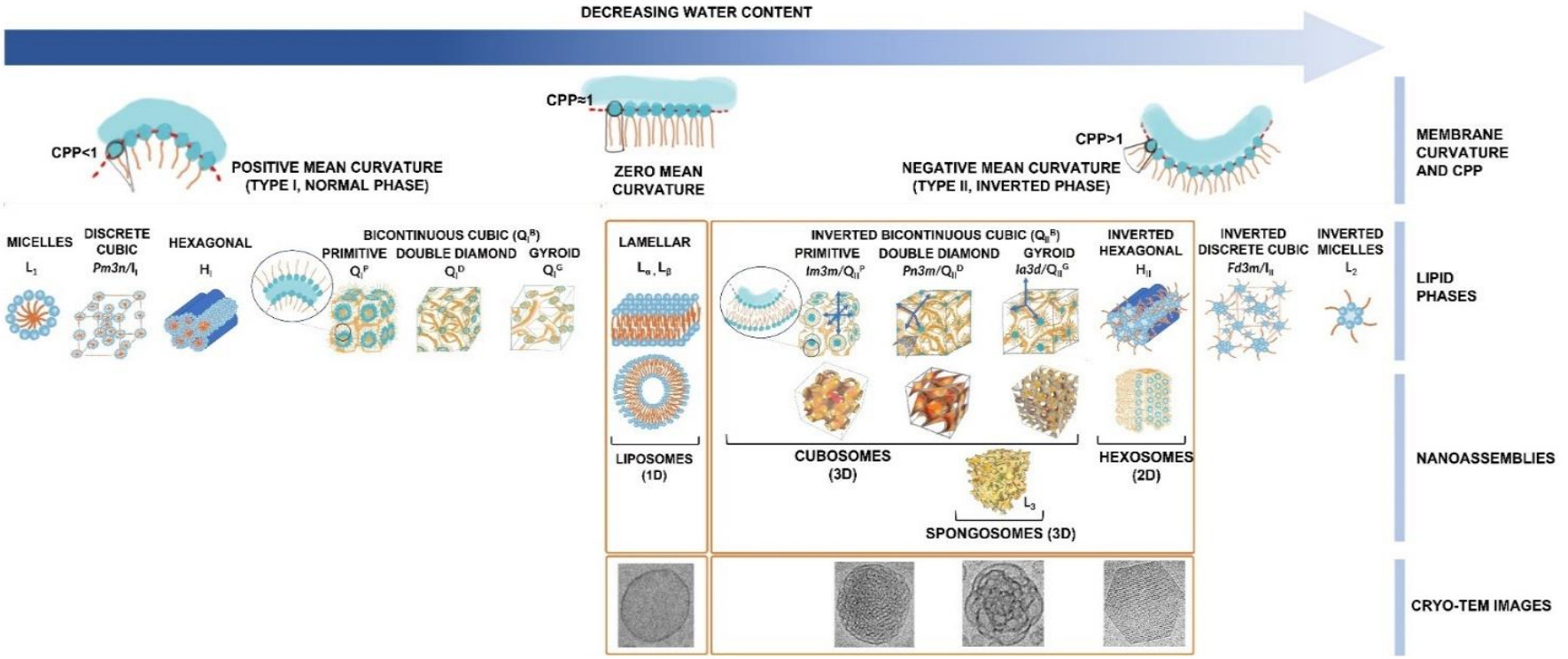

Figure 6: Schematic representation of common lipid mesophases resultant from self-assembled amphiphiles according to their critical packing parameter (CPP) and respective most common LLC nanosystems with their morphologic characterization by cryo-transmission electronic microscopy (cryo-TEM).

Reprinted from Araújo-Silva et al., 2023. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1878, Araújo-Silva et al., Lyotropic liquid crystalline 2D and 3D mesophases: Advanced materials for multifunctional anticancer nanosystems, p. 189011, Copyright (2023), with permission from Elsevier.

Cubosomes are nanosystems known for their exceptional stability, which is primarily attributed to their internal lipid cubic phase structure. In many formulations, an outer polymer corona is added to act as an emulsifier, further enhancing stability by preventing aggregation and maintaining dispersion in the dispersion. These structural features ensure the long-term stability of cubosomes, making them effective for various applications (Seddon et al., 1993).

Cubosomes originate from bicontinuous lipid cubic phases and have a continuous, periodic lattice structure for membranes with pores formed by the intertwining of two water channels (Araújo-Silva et al., 2023; Barriga et al., 2019). Cubic phases are primarily categorized based on the continuity of their water channels into bicontinuous phases and discrete phases. Specifically, bicontinuous cubic phases include normal bicontinuous phases, Q_I^B, with continuous hydrophilic regions and inverted bicontinuous phases, Q_{II}^B, with continuous hydrophobic regions. Conversely, discrete cubic phases, which are less commonly discussed in relation to cubosomes, have separated water channels (Larsson et al., 1999; Angelov et al., 2005). Cubosomes consist of inverted bicontinuous phase Q_{II}^B, this structural configuration comprises a single 3D curved lipid bilayer that separates two continuous hydrophilic regions. These regions intertwine but there is no direct connection between them (Boden et al., 1993).

According to the literature, Q^B phases are classified into three crystallographic space groups: (i) *la3d*, known as the gyroid type, featuring a three-by-three pattern connecting hydrophilic regions; (ii) *Pn3m*, referred to as the double-diamond type, with pairs of water channels organized in a four-by-four tetrahedral network; and (iii) *lm3m*, described as the primitive type, where each water channel is structured in a six-by-six pattern, forming an orthogonal network (Araújo-Silva et al., 2023; Seddon et al., 1993).

Hexagonal phases arise when specific amphiphiles, such as phospholipids (e.g., phosphatidylcholine), glycolipids (e.g., cerebrosides), fatty acids, steroids (e.g., cholesterol), and surfactants (e.g., Tween® 20, Tween® 80), organize into cylindrical structures within an aqueous environment (Mishra et al., 2018, Zhang et al., 2021). Polar lipids that mimic the natural amphiphiles compatible with biological systems have applications in a variety of fields, including food and pharmaceuticals. In water, they naturally form micellar solutions or various types of lyotropic liquid crystals. Higher temperatures induce the formation of reverse hexagonal phases. Examples of such

amphiphiles include GMO, phytanyl glycerate (PG), oleyl glycerate (OG), and PHYT (Araújo-Silva et al., 2023).

The hexagonal phase is made up of 2D hexagonal lattices formed by micellar cylinders developed by lipid self-assembly. Hexosomes are non-lamellar lipid nanosystems based on normal hexagonal (H_I) or on inverted hexagonal (H_{II}) mesophases (Figure 6) (Hirlekar et al., 2010). The H_I phase features cylindrical micelles with the hydrophobic part inward, resulting in a positively curved interface. In contrast, the H_{II} phase consists of hexagonally packed inverted cylindrical micelles that enclose water channels and have a negatively curved interface. H_I is water-dominant, while H_{II} is lipid-dominant (Seddon, 1990). The transition between normal hexagonal and inverse hexagonal mesophases is primarily determined by the polarity of the solvent and the nature of the molecule (Girdhar et al., 2018). This transition offers several advantages, including high drug-loading capacity and improved *in vivo* stability, especially for delivering large molecules like peptides and proteins (Rapalli et al., 2020a). These exceptional attributes of hexosomes make them valuable for enhancing the solubility of drugs with limited aqueous solubility (Hirlekar et al., 2010).

The inverted hexagonal phase (H_{II}) is predominantly encountered when there are small water channels and substantial interfacial curvatures. Reduced water content serves as a mechanism to reduce the radius of water channels, thereby contributing to the stability of the H_{II} phase through lower hydration levels. Several factors that enhance spontaneous curvature, including the presence of unsaturated hydrophobic chains, branching, longer chain lengths, and higher temperatures, further promote the formation of the H_{II} phase. A theoretical model based on the interaction between bending energy and packing constraints is employed to elucidate these phenomena (Drummond & Kaasgaarda, 2006).

Spongosomes are non-lamellar lipid nanosystems produced when an amphiphilic lipid self-assembles in an excess aqueous phase, leading in the development of a liquid crystalline sponge phase (L_3) (Figure 6). These nanosystems exhibit an internal structure with randomly arranged bicontinuous lipid membranes, creating a porous architecture that enhances their capacity to load drugs and other molecules efficiently. To stabilize their surfaces, various materials such as alginate chains are employed, imparting stealth properties and ensuring colloidal stability (Zou, 2017). The sponge phases have a disordered lamellar nanostructure similar to that of a melting cubic mesophase, with irregular 3D membrane topologies composed of inverted bilayers surrounded by a lipid

phase and relatively large aqueous pores (Drummond & Fong, 1999; Hyde, 2001a). When certain raw materials, such as glycerol dioleate (GDO) and diglycerol monooleate (DGMO), are combined in an equal weight proportion and supplemented with a small amount of polyoxyethylene (20) sorbitan monooleate, the resulting mixture, when combined with water, produces exquisite nanosystems with an internal "sponge" mesophase structure and spherical configurations. These complex nanostructures are generated without the use of high-energy dispersion techniques (Barauskas et al., 2005). For internally organized nanosystems that lack a consistent internal arrangement, like sponges in the L_3 mesophase, that cannot be thoroughly revealed using alternative characterization methods, such as small-angle X-ray diffraction, cryogenic transmission electron microscopy (cryo-TEM) offers an exceptional and comprehensive depiction of their structure (Demetzos et al., 2022).

3. Preparation of non-lamellar lipid nanosystems

As referred before, the 2D and 3D LLC nanosystems consist of amphiphilic lipid building blocks that serve as precursors to the bulk cubic, hexagonal, or sponge phases. GMO and PHYT are the most commonly used amphiphilic lipid building blocks (Sadhu et al., 2018). While cubic, hexagonal, and sponge lipid mesophases are thermodynamically stable in bulk form, they tend to aggregate when dispersed in a solvent to produce nanosystems. The stability of 2D and 3D LLC nanosystems can be preserved by coating their surfaces with various stabilizers, such as amphiphilic block copolymers (e.g., Pluronic® derivatives), lipid-copolymers (e.g., polyethylene glycol-based lipids and polysorbate 80), and other steric stabilizers (Zou et al., 2017; Saber et al., 2018)

Different preparation methods for LLC nanosystems have emerged, each with its own set of characteristics and applications. The 'top-down' and 'bottom-up' strategies constitute the core of LLC nanosystems production (Araújo-Silva et al., 2023).

The top-down approach involves mechanically shearing lipid building blocks and stabilizers to prepare bulk phases with cubic, hexagonal, or sponge structures (Sung et al., 2020). Subsequent application of high energy, such as high-pressure homogenization or ultrasonication, transforms these phases into a single thermodynamic phase with periodic liquid crystalline structure. Conversely, the bottom-up method includes the incorporation of a hydrotrope compound, such as ethanol or urea, into the lipid-stabilizer mixture, enabling formation via a dilution method, typically with minimal energy input like vortexing (Gersten et al., 2002). This method is particularly effective in producing smaller and more stable LLC nanosystems, albeit with occasional vesicular structures. Microfluidics offers innovative control over size and scalability, while spray drying yields powdered 2D and 3D LLC nanosystems suitable for various administration routes (Omram, 2001; Hassanpour & Dehghani, 2017). However, spray drying often results in high polydispersity. Additional refinement through a heat treatment process can reduce polydispersity, though it is less suitable for temperature-sensitive drugs (Spicer et al., 2002). These versatile preparation methods establish the foundation for a wide range of LLC nanosystems, each with unique characteristics ready for exploration in diverse biomedical and drug delivery applications (Barriga et al., 2019).

To incorporate drugs into LLC nanosystems, lipophilic and hydrophilic compounds are dissolved in the bulk mesophase before dispersion. Lipophilic drugs tend to localize in

the hydrophobic regions of nanostructures, while hydrophilic drugs are predominantly found in water channels. High-energy procedures like high-pressure homogenization and ultrasonication are used in the top-down methodology for drug-loaded LLC nanosystems. An alternative approach utilizes a shear device based on a Couette cell, limiting heat energy input and producing monodisperse LLC nanosystems with a high concentration of lipophilic molecules. This method has also been applied successfully for hydrophilic drugs (Duttagupta et al., 2016). The bottom-up approach combines amphiphiles with hydrotropes before dilution, ultimately producing more stable nanosystems with controlled aqueous addition (Tilekar et al., 2014).

Both techniques may yield not only LLC nanosystems but also vesicles, which could affect drug release. To address this, three purification methods are available. Heat treatment (usually above 120°C) purifies cubosomes, producing ordered cubic particles from smaller vesicles, albeit with limited applicability in drug delivery (Araújo-Silva et al., 2023). The modified dialysis technique, which combines the bottom-up approach and dialysis, obtains a preparation devoid of hydrotropes and micelles without using high-energy processes. The premix membrane emulsification method repeatedly extrudes a pre-dispersed emulsion through a membrane with desired pore sizes, facilitating the production of monodisperse nanosystems (Sung et al., 2020). Recent advancements demonstrate that altering solution characteristics like pH or ionic strength can influence the formation and stability of cubosomes or hexosomes without requiring high-energy input, allowing for the design of desired nanostructures. For instance, cubosome dispersion can be achieved by introducing phosphate buffer to an aqueous solution of cationic vesicles (Spicer et al., 2002). Additionally, modifications in the content of ionic surfactants or in the ionic strength of the solution can also cause nanostructures to transition, enabling the detection of specific toxic ions. These versatile preparation and purification methods provide researchers with a toolkit for designing and optimizing LLC nanosystems tailored for various drug delivery applications (Araújo-Silva et al., 2023; Popescu et al., 2015; Lancelot et al., 2014).

When compared to liposomes, the non-lamellar liquid crystalline phases have a well-organized internal structure and higher lipid volume per nanosystem, providing a substantial lipophilic surface area for accommodating hydrophobic drugs. Furthermore, their compartmentalizing properties, which are unique to these phases, involve mono- or bicontinuous systems containing with both hydrophilic and hydrophobic regions capable

of solubilizing hydrophobic, hydrophilic, and amphiphilic compounds. Consequently, cubic and hexagonal phases have gained considerable recognition as drug delivery nanosystems in various fields, including pharmaceuticals, agrochemicals, food, and cosmetics (Mishra et al., 2018). Non-lamellar lipid nanosystems can be tailored to provide prolonged drug-release patterns and to hinder the rapid *in vivo* degradation of sensitive therapeutic agents. The non-lamellar lipid nanosystems demonstrate remarkable stability against phase changes, particularly under conditions of high hydration. This inherent stability is advantageous for the practical application of cubosomes and hexosomes. Moreover, the structural characteristics of these nanosystems can be readily adjusted by manipulating processing variables, such as pressure, temperature, amphiphile concentration, and the inclusion of additives (Mishra et al., 2018).

Besides lipid mesophases have interesting properties for drug delivery, cubosomes, hexosomes, and spongosomes, each present their own set of limitations. One significant challenge is the limitation of large-scale production methods, which can impede their widespread application (Zhai et al., 2019).

4. Applications of non-lamellar lipid nanosystems

Hybrid lipid-polymer nanosystems, consisting of 2D and 3D LLC mesophases, often referred to as exotic lipid phases, offer promise as advanced delivery systems. These non-lamellar lipid nanosystems, characterized by high curvature, offer advantages such as an increased surface area-to-volume ratio. Additionally, these structures can be tailored to vary membrane stress, which enhances permeation and cell internalization, with the added benefit of tunable membrane curvature and lipid pores (Barriga et al., 2019; Garg et al., 2007). Furthermore, the resemblance to the liquid crystal structures of intercellular lipids in SC improves contact with the skin and mucosa, boosting the potential for topical drug delivery efficiency (Gazga-Urioste et al., 2019).

The following are some examples of studies published in the literature on the development of non-lamellar lipid nanosystems for use via the topical administration routes investigated in this dissertation, namely skin, ocular, and vaginal administration (Table I).

4.1. Skin drug delivery

Hexosomes and cubosomes, two types of non-lamellar lipid nanosystems with distinct structures, are particularly useful for enhancing therapeutic agent topical permeation.

Hexosomes, with their hexagonal phase, have been efficiently utilized to vehicle cyclosporin A (CysA), a model peptide, in both *in vitro* and *in vivo* studies on mice skin, reinforcing the topical delivery of peptides without inducing skin irritation (Lopes et al., 2006). In *in vitro* studies, using porcine ear skin mounted in a Franz diffusion cell, hexosomes promoted CysA skin permeation, achieving approximately 2-fold higher concentrations in the SC and in the epidermis (excluding SC) plus dermis (E+D) compared to a control formulation (olive oil). In *in vivo* studies with hairless mice revealed 1.5- and 2.8-times higher concentrations in the SC and in the E+D, respectively, when hexosomes were employed. After two days of topical administration, hairless mice's skin was histologically examined to assess potential skin irritation. There was no evidence of epidermal thickening, edema, or immunocyte infiltration, indicating that the hexosomes did not induce local irritation. This enhanced delivery system shows promise

for the effective and safe topical administration of CysA, potentially improving therapeutic outcomes for skin conditions that require such treatment.

In another study, Gazga-Urioste et al. (2018) also investigated the potential of ketoconazole-loaded hexosomes for topical application. Hexosomes, characterized by their hexagonal lipid packing, provide enhanced stability and controlled drug release kinetics. This formulation approach aims to ensure sustained delivery of ketoconazole, an antifungal agent, to the affected skin areas, thereby improving treatment outcomes for fungal infections while minimizing the frequency of application.

Cubosomes have also shown considerable potential for improving therapeutic efficacy across various routes of administration, particularly topical skin application. For instance, Morsi et al. (2014) emphasized the topical use of silver sulfadiazine (SSD)-loaded cubosomes, which were incorporated into hydrogels, for managing infected burns, which resulted in faster tissue recovery compared to conventional cream administration while minimizing cytotoxicity. The authors reported that SSD-loaded cubosomes enhanced wound healing through improved drug retention and penetration in the skin layers, leading to more effective bacterial eradication and lower inflammation. Histological analysis confirmed that SSD-loaded cubosomes did not induce adverse skin reactions, making them a safe and efficient treatment for infected burns.

Similarly, Salah et al. (2017) demonstrated the potential of transdermal cubosomes for enhancing the bioavailability of etodolac through skin application. Their study showed that etodolac-loaded cubosomes provided extended drug delivery and improved pharmacokinetic profiles compared to oral capsules. The *in vitro*, using excited mice skin, and *in vivo*, involving healthy human volunteers, studies revealed significantly higher concentrations of etodolac in the SC and deeper skin layers when administered via cubosomes, resulting in prolonged therapeutic effects and reduced systemic side effects. The cubosomes also maintained the structural integrity of the skin barrier and did not cause irritation, as confirmed by histopathological evaluations.

In another study, Peng et al. (2015) reported that capsaicin-loaded cubosomes outperformed conventional creams in terms of sustained skin retention and targeted delivery. The study demonstrated that capsaicin-loaded cubosomes provided prolonged pain relief and reduced the frequency of application needed, enhancing patient comfort and compliance. The enhanced skin retention was attributed to the cubosomes' ability to

penetrate deeper skin layers and release the drug in a controlled manner, avoiding the initial burst release often seen with conventional creams. No significant skin irritation or adverse effects were observed, highlighting the potential of cubosomes for the effective and safe delivery of capsaicin.

Janakiraman et al. (2019) explored the potential of methotrexate (MT)-loaded cubosomes as a topical treatment for rheumatoid arthritis, aiming for controlled drug release and reduced dosing frequency. MT-loaded cubosomes were developed to alleviate pain and joint stiffness by maintaining stable concentrations of MT at targeted sites through non-invasive skin application, which enhances drug permeation. The formulation employed the non-ionic surfactant, poloxamer 188, to facilitate MT permeation, ensuring efficient delivery of drug to affected areas. *In vitro* release studies demonstrated a sustained release pattern of MT from cubosomes over 12 hours, indicating prolonged therapeutic action. *Ex vivo* skin permeation research on excised Wistar albino rat skin confirmed significant MT permeation without causing skin irritation. Histopathological evaluations and fluorescent microscopic analysis further validated the superior permeation ability of cubosomes, underscoring their potential efficacy in clinical applications. Additionally, thermal stimulus time (TST) studies revealed enhanced analgesic effects of MT-loaded cubosomes compared to standard diclofenac gel, suggesting improved pain relief benefits. In an animal model of complete freund's adjuvant (CFA)-induced arthritis, MT-loaded cubosomes treatment significantly reduced inflamed paw thickness from 1.47 cm on day 1 to 1.03 cm on day 15, demonstrating their promise as an effective treatment for rheumatoid arthritis upon topical application.

Esposito et al. (2005) explored the potential of cubosomes to enhance the percutaneous absorption of indomethacin (IND), a nonsteroidal anti-inflammatory agent. The authors focused their study on optimizing drug delivery through the skin barrier, with the aim of achieving therapeutic concentrations locally while minimizing systemic exposure and associated side effects. Cubosomes offered controlled release of IND, which could prolong therapeutic action and improve patient adherence to treatment regimens.

Furthermore, Rapalli et al. (2023) highlighted the application of non-lamellar lipid nanosystems loaded with apremilast, emphasizing their enhanced skin permeation and biocompatibility. Apremilast is a promising therapy for psoriasis, which is a chronic inflammatory skin condition. The non-lamellar lipid nanosystems demonstrated superior drug delivery efficiency by facilitating deeper penetration into the skin layers, which is

crucial for targeting inflammatory pathways associated with psoriatic lesions. This approach not only enhances therapeutic efficacy but also reduces potential systemic side effects compared to conventional oral administration.

All the studies presented demonstrate the versatility and efficacy of non-lamellar lipid nanosystems in advancing topical drug delivery approaches to the skin for the treatment of various skin/cutaneous conditions, proving effective vehicles for the delivery of a wide range of therapeutic agents, from anti-inflammatory agents to antifungals, and for the treatment of chronic skin disorders like psoriasis.

4.2. Ocular drug delivery

Non-lamellar lipid nanosystems have also shown considerable promise for enhancing ocular drug delivery. These sophisticated nanosystems improve drug penetration, stability, and bioavailability, optimizing therapeutic outcomes for a variety of eye conditions. For example, the incorporation of travoprost into non-lamellar lipid nanosystems has resulted in a safe and effective approach for glaucoma treatment (El-Gendy et al., 2023). In this study, the authors reported that the nanostructures, encapsulating travoprost, allow for superior drug penetration through the corneal layer, while maintaining high stability and travoprost entrapment efficiency. Travoprost-loaded non-lamellar lipid nanosystems significantly enhances the therapeutic efficacy of travoprost in reducing intraocular pressure. The effectiveness of travoprost encapsulated in these nanostructures was compared to Travatan®, a commercially available formulation. The nanostructures demonstrated enhanced permeation properties, ensuring sustained and effective delivery of travoprost to targeted ocular tissues. This comparative analysis highlights the potential of non-lamellar lipid nanosystem to improve existing formulations, offering enhanced therapeutic outcomes for managing intraocular pressure in conditions like glaucoma.

Furthermore, hexosomes loaded with pilocarpine nitrate (PN) demonstrated prolonged efficacy in reducing intraocular pressure in rabbits, outperforming both commercial pharmaceutical products containing PN and physiological saline in terms of sustained therapeutic effects (Li et al., 2013). Commercial products typically include formulations like eye drops, which are commonly used to treat conditions such as glaucoma and ocular hypertension. Li et al. (2013) highlighted that the sustained release properties of PN-loaded hexosomes not only prolonged the duration of therapeutic action, but also

enhanced the drug's bioavailability in ocular tissues. This study underscores the potential of hexosomal formulations as advanced delivery systems for improving the management of intraocular pressure disorders, offering advantages over traditional dosage forms in ophthalmic treatments.

Cubosomes containing flurbiprofen (FB) exhibit minimal ocular irritation, as confirmed by the Draize method and histological examination (Han et al., 2010). The Draize method is a standardized test used to evaluate the potential irritancy of chemicals or formulations when applied to the eyes or skin of experimental animals, typically rabbits in ocular studies. This method involves grading the severity of various types of irritation, including redness, swelling, and discharge, over a specified period following application. Histological examination was critical in confirming the minimal ocular irritation caused by FB-loaded cubosomes (Han et al., 2010). Histological observations involve microscopic analysis of tissue samples from the eye after exposure to the formulation, assessing changes in tissue structure, inflammation, and cellular responses, providing insights into the biological effects of the test substance. The authors reported that cubosomes enhanced transcorneal FB permeation, resulting in significantly higher Area Under the Curve (AUC) and Mean Residence Time (MRT) values than FB sodium (FB Na), a salt form of FB used in ophthalmic formulations (eye drops) to treat inflammation and reduce intraocular pressure. This combination of enhanced transcorneal permeation and minimal ocular irritation suggests that FB-loaded cubosomes could potentially offer better ocular tolerability and efficacy compared to traditional FB Na eye drops, highlighting their promise for ocular drug delivery applications.

Additionally, gatifloxacin (GTX)-loaded cubosomes have attained remarkable efficacy in ocular applications (Nasr et al., 2022). These authors reported higher corneal permeation and a fourfold reduction in the minimum inhibitory concentration (MIC) against clinically isolated methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The data suggest that cubosomes are effective in treating bacterial infections of the eye. GTX in conventional dosage forms, such as eye drops or ointments, is commonly used to treat ocular infections. However, the study conducted by Nasr et al. (2022) highlights the superiority of cubosomes in enhancing GTX delivery and efficacy. Cubosomes provide advantages over traditional formulations by improving corneal drug permeation and reducing MIC values, indicating their potential to improve treatment outcomes in ocular bacterial infections. In another study, dexamethasone (DEX) loaded in cubosomes

significantly enhances drug permeability and retention in the preocular region, improving ocular DEX bioavailability and ensuring extended therapeutic effects (Gan et al., 2010). Moreover, non-lamellar lipid nanosystems containing tetrandrine (TET) show promise for increasing ocular bioavailability by enhancing retention time and corneal permeability (Liu et al., 2016). The *in vitro* release studies revealed a sustained TET release profile. A corneal permeation study demonstrated that the apparent permeability coefficient of the non-lamellar lipid nanosystems was 2.03-fold higher than that of the TET solution (free drug solution). Furthermore, a pre-ocular retention capacity analysis indicated that non-lamellar lipid nanosystems had significantly longer retention than TET solution. Additionally, a pharmacokinetic study conducted in rabbit aqueous humors showed that TET-loaded non-lamellar lipid nanosystems exhibited 2.65-fold higher ocular TET bioavailability compared to the TET solution. Finally, cubosomes containing timolol maleate has demonstrated the ability to increase corneal permeability and bioavailability, indicating substantial potential for treating ocular diseases (Huang et al., 2017).

All the studies presented suggest that non-lamellar lipid nanosystems are a promising approach to enhance drug penetration across ocular membranes, stability, and bioavailability, making them highly efficient for diverse ocular applications.

4.3. Vaginal drug delivery

Non-lamellar lipid nanosystems have shown remarkable drug delivery potential in vaginal applications. For instance, Aboud et al. (2018) investigated the use of cubosomes in situ gelling sponges (CIS) for intravaginal administration of sildenafil citrate (SIL), i.e., a type 5-specific phosphodiesterase inhibitor that is use in the management of infertility in women. Their study demonstrated that intravaginal administration of CIS significantly increased endometrial thickness, with notable congestion and dilatation of endometrial blood vessels, as compared to both intravaginal free SIL in situ gelling sponges (FIS) and oral-treated groups. Furthermore, the *in vivo* pharmacokinetic study revealed that intravaginal administration of CIS resulted in significantly lower maximum concentration (Cmax) and AUC of SIL in plasma, alongside a prolonged mean elimination half-life, when compared to oral administration of SIL solution. In another study, Victorelli et al. (2022) examined the use of cubosomes for the intravaginal delivery of curcumin in the treatment of cervical cancer in order to increase its bioavailability and

local absorption. The authors found that curcumin released from the cubosomes was retained in the vaginal epithelium, indicating potential for topical administration. *In vitro* cytotoxicity and cellular uptake assays demonstrated that HeLa cells could internalize the cubosomes, enhancing curcumin's effect against cervical cancer cells. Additionally, Li et al. (2016) explored the use of LLC nanosystems to deliver protein therapeutics like interferon vaginally. Their research highlighted the stability, ease of loading, and controlled release of protein therapeutics using LLC nanosystems. The *in vitro* and *in vivo* mucoadhesive properties were assessed to determine the ability of LLC nanosystems to adhere to the vaginal mucosa, which is crucial for localized drug delivery. Microbiological safety evaluations ensured that LLC nanosystems did not disrupt the natural vaginal flora that is essential for maintaining vaginal health. Histological examinations and histopathological analysis of vaginal tissue samples provided insights into any microscopic changes, such as inflammation or tissue damage, caused by the LLC nanosystems. Therefore, the authors suggested that LLC nanosystems are a promising mucoadhesive drug delivery system for safe vaginal administration of protein therapeutics, offering controlled release and localized therapeutic benefits.

The research described here indicate that nanosystem-based delivery atrategies have the potential to enhance the localized effects of various therapeutic agents in vaginal treatments. By utilizing nanosystems, such as non-lamellar lipid nanosystems, therapetuics agents can be targeted directly to the vaginal mucosa, improving their efficacy and reducing the required dosage. This targeted delivery approach not only enhances therapeutic outcomes but also minimizes systemic exposure, thereby reducing the likelihood of systemic side effects. The controlled release capabilities of non-lamellar lipid nanosystems further contribute to maintain sustained therapeutic levels at the site of action, ensuring prolonged benefits. Overall, nanosystem-based delivery systems represent a promising strategy for optimizing vaginal drug treatments, offering enhanced efficacy with reduced systemic risks.

Table I: Examples of non-lamellar lipid nanosystems applications in topical routes of administration, namely skin, ocular and vaginal.

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
Hexosomes	CysA	*In vitro* (porcine ear skin) and *in vivo* studies (mice) / Skin administration	GMO Poloxamer Oleic acid	Top-down approach	Size: 181.77±1.08 PDI: N.R.	N.R.	EE: 94.3±1.2	• In both *in vitro* and *in vivo* studies: hexosomes significantly enhanced CysA skin penetration compared to the control formulation using olive oil • *In vitro* studies: CysA-loaded hexosomes significantly increased CysA concencentration in the SC after 6 hours (p<0.05) and 12 hours (p<0.01) post-application. The combined epidermis (excluding SC) and dermis (E+D) also revealed significant increases CysA concentrations after 6 and 12 hours (p <0.05) • *In vivo* studies: hexosomes achieved 1.5 and 2.8 times greater concentrations in the SC and E+D, respectively, at 6 hours after topical administration. The maximum concentrations of CysA in both the SC and E+D were approximately two-fold higher with hexosomes compared to the control formulation	Lopes et al., 2006
Hexosomes	Ketoconazole	*In vitro* studies / Skin administration	GMO P407 Propylene glycol monolaurate	Top-down approach	Size :107±20	+ 4.45±0.50	EE: 85.98±3.77	• Hexosomes released ketoconazole in a controlled behavior described by a first order kinetics • Hexosomes revealed physicochemical stability and performance under typical storage conditions	Gazga-Urioste et al., 2018

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
Cubosomes	SSD	*In vitro* and *in vivo* (Wister rats) studies / Skin administration (topical treatment of infected burns)	GMO P407 PVA (0 to 5%) (stabilizer)	Top-down approach	Size: from 152.3±1.4 to 398.6±4.37 PDI:0.25±0.004 to 0.65±0.45	N.R.	EE: 86.04 to 94.56	• SSD-loaded cubosomes decreased the cytotoxic effect of silver by controlling the release of SSD and decreasing the dose of SSD to 0.2% (fifth that of the market product Dermazin®) • Tissue healing began on day 9, which was 6 days before the commercial cream (day 15)	Morsi et al., 2014
Cubosomes	Etodolac	*In vitro* (excited mice skin) and *in vivo* (healthy human volunteers) studies / Skin administration	GMO P407 (ratio: 2)	Top-down approach	Size: 135.95 to 288.35 PDI : N.R.	-18.40 to -36.10	EE: 100 DL: 1.28 to 6.09	• Cubosomes loaded with etodolac penetrated mice's skin quickly, followed by slower etodolac penetration lasting up to 24 hours • Pharmacokinetic study in human volunteers: etodolac-loaded cubosomes enhanced drug relative bioavailability as compared to the oral capsules (266.11%, $p<0.05$) with longer half-life and higher MRT (at 18.86 and 29.55 h, respectively)	Salah et al., 2017
Cubosomes	Capsaicin	*In vitro* (abdominal skin of excised Sprague Dawley rats) and *in vivo* (mouse skin) / Skin administration	F1: PHYT P407 F2: GMO P407	Top-down approach	F1: Size: 251.3 PDI: 0.093 F2 Size: 215.7 PDI: 0.099	NR	F1: EE: 97.14±0.54 DL: N.R. F2: EE: 97.58±0.53	• Percutaneous absorption of capsaicin from the GMO-based cubosomes was lower than that of the conventional cream • Cubosomes produced higher and sustained skin retention of capsaicin than the cream due to the delayed drug diffusion into the skin • Skin-targeted, sustained, and thermodynamically stable characteristics of cubosomes provided an interesting system for the topical delivery of capsaicin	Peng et al., 2015
Cubosomes	MT	*In vitro*, *ex vivo* (wistar albino rat	Poloxamer 188	Top-down approach	Size: 626.13±14.15	-6.29±1.07	EE: 92.94±0.9	• MT-loaded cubosomes had a higher anti-inflammatory efficacy of 11.9% compared to diclofenac sodium's (10.4%)	Janakiraman et al., 2019

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
		skin), and *in vivo* (wistar albino rats) studies / Skin administration	Cetyl palmitate		PDI: N.R.			• *Ex vivo* skin permeation analysis: 2.50 ± 0.3 ng of MT permeated within 2 hours and increased to 8.80 ± 5.2 ng within 12 hours, without causing skin irritation • Size of inflamed paw in the animal group treated with MT-loaded cubosomes significantly reduced from 1.47 cm on day 1 to 1.03 cm on day 15, as demonstrated by animal imaging system results	
Cubosomes	IND	*In vitro* (samples of adult human skin from breast reduction operations) and *in vivo* (human volunteers) studies / Skin administration	GMO P407	Top-down approach	Size: 206.7±0.5 PDI: 0.13±0.02	N.R.	EE:99.8±0.1	• Cubosomes controlled percutaneous absorption of IND • *In vivo* studies: cubosomes drastically influenced the lasting of anti-inflammatory activity and consequently IND prolonged release	Esposito et al., 2005
Non-lamellar lipid nanosystems	Apremilast	*In vitro* (immortal keratinocyte cells), *ex vivo* (goat ear skin) and *in vivo* studies (Swiss albino mice) / Skin administration	GMO Labrafil® M 2125 CS P407	Top-down approach	Size: 173.25±2.192 PDI: 0.273±0.008	N.R.	EE: 75.028±0.235	• Non-lamellar lipid nanosystems demonstrated a prolonged release of apremilast for 18 h • *Ex vivo* studies: non-lamellar lipid nanosystems enhanced skin drug retention up to 3.2 and 11.9-fold higher, in SC and viable epidermis compared to conventional gel preparation • Non-lamellar lipid nanosystems demonstrated non-toxic in cell viability studies, and did not cause irritation or erythema in *in-vivo* skin irritation tests • Non-lamellar lipid nanosystems loaded gel confirmed improved both permeation and	Rapalli et al., 2023

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
								retention of apremilast compared to conventional gel	
Non-lamellar lipid nano-systems	Travoprost	*In vitro*, *ex vivo* (excised rabbit corneas) and *in vivo* (New Zealand white rabbits) studies / Ocular administration	GMO PHYT Tween® 80 (stabilizer agent) Oleic acid or Captex® 8000 (penetration enhancer)	Top-down approach	Size: 109.08±6.53 to 346.45±30.05 PDI: 0.15±0.04 to 0.61±0.07	-15.18±0.95 to -81.03±7.71	EE: 82.54±7.65 to 85.30±4.29	• Non-lamellar lipid nanosystems improved drug penetration throughout the corneal layer, stability, and high travoprost entrapment efficiency • Travoprost's bioavailability was threefold higher than when delivered from the market pharmaceutical product Travatan® • Non-lamellar lipid nanosystems showed reductions in intraocular pressure lasting longer compared to Travatan® • Non-lamellar lipid nanosystems showed no evidence of eye damage compared to the control eye	El-Gendy et al., 2023
Hexosomes	PN	*In vitro*, *ex vivo* (excised rabbit corneas), and *in vivo* (rabbits) studies / Ocular administration	GMO P407	Top-down approach	Size: 202.28±19.32 PDI: 0.11 ± 0.01	N.R.	EE: 57.1±4.4 DL: 6.0±1.0	• Hexosomes demonstrated a controlled PN release profile • *Ex vivo* study : apparent permeability coefficient of PN-loaded hexosomes was 2.05-fold higher than that of commercial eye drops • PN-loaded hexosomes had a prolonged effect on decreasing intraocular pressure of rabbits compared to commercial products containing PN and physiological saline solution	Li et al., 2013
Cubosomes	FB	*In vitro* and *in vivo* (rabbits eye) studies	GMO P407	Top-down approach	Size: 148.6±2.16	-13.59±1.37	EE: 99.38±0.022	• Cubosomes exhibited low ocular irritating properties as evaluated by both the Draize method and histological examination	Han et al., 2010

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
		/ Ocular administration	(ratio = 9)		PDI: 0.132±0.010			• *In vitro* corneal penetration assessment: cubosomes enhanced the transcorneal FB permeation • *In vivo* studies: FB-loaded cubosomes had higher AUC and MRT values than FB Na eye drops • Cubosomes are low-irritant vehicle for the effective ocular FB delivery	
Cubosomes	GTX	*Ex vivo* (rabbits' corneas) and i*n vivo* studies (Wistar rats) / Ocular administration	GMO P407	Top-down approach	Size: 197.46 ± 9.40 PDI: 0.14 ± 0.05	-21.90 ± 2.03	EE: 52.8±2.93	• GTX-loaded cubosomes had higher corneal permeation (≈ 1.3 times) and a fourfold reduction in the MIC against clinically isolated MRSA strain compared to GTX aqueous dispersion • Histological structures of corneal tissues from rats treated with GTX-loaded cubosomes did not present difference from those of normal rat corneas.	Nasr et al., 2022
Cubosomes	DEX	*In vitro* and *ex vivo* (excised rabbit corneas) and *in vivo* (rabbits) studies / Ocular administration	GMO P407 (ratio: 9)	Top-down approach	F1 (10% oil) Size: 214.1±41.1 PDI: 0.144±0.021 F2 (20% oil) Size: 226.3±55.6	N.R.	F1 EE: 98.8±2.6 F2 EE: 98.5±5.4	• Permeability coefficient of DEX-loaded cubosomes was significantly enhanced compared to DEX sodium phosphate eye drops • F1 cubosomes with low viscosity were retained in the preocular region for longer time compared to solution and carbopol gel containing DEX • Ocular bioavailability of DEX has improved • Both F1 and F2 cubosomes had no deleterious influence on corneal structure and integrity in *in vitro* ocular tolerance test	Gan et al., 2010

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
					PDI: 0.176±0.014				
Cubosomes	TET	*Ex vivo* (excised rabbit corneas) and *in vivo* (rabbits) studies / Ocular administration	GMO P407	Top-down approach	Size: 170.0±13.34 PDI: 0.166±0.02	+29.3±1.25	EE: 95.46±4.13 DL: 1.63±0.07	• *Ex vivo* studies: higher corneal permeability and longer TET pre-ocular retention compared to TET solution • Cubosomes increased the ocular bioavailability of TET by enhancing its retention time and permeation into the cornea	Liu et al., 2016
Cubosomes	Timolol maleate	*Ex vivo* (excised rabbit corneas) and *in vivo* (rabbits) studies / Ocular administration	GMO P407 (ratio: 9)	Top-down approach	Size: 142 nm PDI: 0.107	-6.27±1.3	EE: 86.4±4.6 DL: 4.1±0.5	• Cubosomes increased the corneal permeability and bioavailability of timolol maleate • *Ex vivo* study: total amount of timolol maleate-loaded cubosomes penetrated was higher compared to commercially eye drops • *In vivo* studies: timolol maleate-loaded cubosomes reduced the IOP in rabbits and had a longer retention time and better lower-IOP effect than the commercial eye drops containing timolol maleate • Neither cytotoxicity nor histological impairment in the rabbit corneas was detected	Huang et al., 2017
Cubosomes	SIL	*In vivo* (female Wistar rats) studies / Intravaginal administration	GMO P407 Polyvinyl alcohol	Top-down approach	Size: 150.81 to 446.02 PDI: 0.01 to 0.21	N.R.	EE: 32.15 to 72.01	• Cubosomes demonstrated a sustained SIL release over 8 h • Intravaginal administration of CIS resulted in increased endometrial thickness with congestion and dilatation of the endometrial blood vessels compared to intravaginal FIS or oral-treated groups • Pharmacokinetic study: significantly lower plasma Cmax and AUC of SIL with a longer	Aboud et al., 2018

Type of non-lamellar lipid nano-systems	Therapeutic / diagnostic agent(s)	Study type / route of administration (or intended route of administration)	Composition (lipid: stabilizer and other additives)	Production technique	Physical-chemical characterization			Relevant outcomes	Ref.
					Size (nm) and PDI	ζ-potential (mV)	EE (%) and DL (%)		
								mean elimination half-life following intravaginal administration of the CIS compared to oral administration of free SIL	
Cubosomes	Curcumin	*In vitro* (HeLa cell line), *ex vivo* and *in* vivo (CAM model) studies / Intravaginal administration (cervical cancer)	GMO DOTAP Pluronic F127 Acetate buffer	Top-down approach	Size: 182±1.8 PDI: 0.27±0.03	42 ± 1.2	EE:86	• Curcumin released from the cubosomes was retained in the vaginal epithelium • *In vitro* cytotoxicity and cellular uptake assays: Hela cells were able to internalize the cubosomes; cubosomes enhanced the curcumin's effect against cervical cancer cells • *In vivo* study. curcumin-loaded cubosomes demonstrated antiangiogenic activity, decreasing the number and diameter of blood vessels	Victorelli et al.., 2022
Non-lamellar lipid nano-systems	Protein therapeutic (Interferon)	*In vitro* and *in vivo* (female rats) studies / Vaginal administration	GMO P407	Top-down approach	Size: 123.5 nm PDI: 0.050	N.R.	EE: 90%	• Interferon-loaded non-lamellar nano-systems exhibited good stability, easy loading, and controlled release • *In vitro* and *in vivo* mucoadhesive properties and local safety of non-lamellar lipid nanosystems	ChunYan Li et al., 2016

List of abbreviations and symbols: **ζ-potential**, zeta potential; **AUC**, area under the curve; **CAM**, chick embryo chorioallantoic membrane; **CIS**, cubosomal *in situ* gelling sponges; **Cmax**, maximum concentration; **CysA**, cyclosporin A; **DEX**, dexamethasone; **DL**, drug loading; **DOTAP**, dioleoyl-3-trimethylammonium propane; **E+D**, epidermis (excluding SC) and dermis; **EE**, entrapment efficiency; **FB**, flurbiprofen; **FB Na**, flurbiptofen sodium; **FIS**, free *in situ* gelling sponges, **GMO**, glyceryl monooleate; **GTX**, gatifloxacin; **IND**, indomethacin; **IOP**, increased intraocular pressure, **MIC**, minimum inhibitory concentration; **MRT**, mean residence time; **MRSA,** methicillin-resistant *Staphylococcus aureus*; **MT**, methotrexate; **N.R.**, not reported; **P407**, poloxamer 407; **PDI**, polydispersity index; **PHYT**, phytantriol; **PN**, pilocarpine nitrate; **SC**, *stratum corneum*; **SSD**, silver sulfadiazine; **SIL**, sildenafil citrate; **TET**, tetrandrine

5. Discussion

Non-lamellar lipid nanosystems, including cubosomes, hexosomes, and spongosomes, form unique nanostructures characterized by their distinct geometric shapes and internal arrangements. These structures can encapsulate both hydrophilic, hydrophobic and amphiphilic therapeutic agents, making them versatile in drug delivery applications. The interest in non-lamellar lipid nanosystems arises from their potential to overcome the limitations of conventional drug delivery systems, such as poor aqueous solubility, instability, and limited bioavailability.

Effective drug delivery systems are essential for achieving optimal therapeutic results. Non-lamellar lipid nanosystems offer the potential to enhance drug delivery by improving solubility, protecting drugs from degradation, and providing controlled release. Their ability to target specific tissues and cells can also reduce systemic side effects, making treatments safer and more effective.

The purpose of this study is to evaluate the efficacy of non-lamellar lipid nanosystems in topical administration, particularly for skin, ocular, and vaginal drug delivery. Building on the selection criteria, which included both recent publications and older studies, this discussion examines the distinct applications of non-lamellar lipid nanosystems in each defined route of administration, considering the benefits and limitation of such nanosystems.

Firstly, studies have demonstrated that non-lamellar lipid nanosystems enhance skin penetration and drug release, making them valuable in dermatology. Hexosomes have effectively delivered CysA without causing skin irritation. Cubosomes have demonstrated promise in various applications, such as SSD for faster recovery in burns. These nanosystems also enhance the bioavailability and drug delivery for etodolac and MT. Additionally, non-lamellar lipid nanosystems containing apremilast improved skin permeation for psoriasis treatment. These findings highlight the potential of these lipid-based nanosystems to improve topical drug delivery.

Secondly, in ocular drug delivery, non-lamellar lipid nanosystems have proven to enhance drug penetration, stability, and bioavailability. Travoprost-loaded non-lamellar lipid nanosystems improved glaucoma treatment by enhancing drug penetration across the cornea. Hexosomes loaded with PN prolonged the reduction of intraocular pressure

in rabbits. FB-loaded cubosomes caused low ocular irritation and enhanced transcorneal permeation. Cubosomes containing gatifloxacin achieved higher corneal permeation and reduced the risk of resistant bacterial strains. Cubosomes also improved the permeability and retention of DEX and increased the bioavailability of Timolol Maleate. Non-lamellar lipid nanosystems loaded with TET boosted ocular bioavailability by increasing retention time and corneal permeation. In summary, these studies collectively highlight the efficacy of non-lamellar lipid nanosystems in ocular drug delivery, offering enhanced drug penetration, sustained release, and improved bioavailability for better therapeutic outcomes.

Finally, non-lamellar lipid nanosystems, such as cubosomes, have shown significant potential for enhancing vaginal drug delivery. Cubosomes demonstrated a sustained release of SIL over an 8-hour period, and intravaginal administration of CIS led to increased endometrial thickness with notable congestion and dilatation of endometrial blood vessels compared to both intravaginal FIS and oral-treated groups . Pharmacokinetic studies indicated a significantly lower plasma Cmax and AUC of SIL, along with a longer mean elimination half-life following intravaginal administration of CIS, compared to oral administration of free SIL, as reported by Aboud et al. (2018). Furthermore, curcumin released from cubosomes was retained in the vaginal epithelium, and in vitro studies showed that Hela cells could internalize cubosomes, enhancing curcumin's efficacy against cervical cancer cells. In vivo, curcumin-loaded cubosomes exhibited antiangiogenic activity, reducing the number and diameter of blood vessels (Victorelli et al., 2022). Additionally, interferon-loaded non-lamellar nanosystems exhibited excellent stability, easy loading, and controlled release, along with favorable in vitro and in vivo mucoadhesive properties and local safety, according to ChunYan Li et al. (2016). These findings collectively underscore the potential of non-lamellar lipid nanosystems in achieving effective, controlled, and localized drug delivery within the vaginal environment.

During my research, I did not find studies that investigated the use of spongosomes for cutaneous, ocular, or vaginal administration. However, spongosomes have been explored as drug delivery carriers as demonstrated in several studies. For example, Zou et al. (2017) demonstrated that spongosomes loaded with Brucea javanica oil (BJO) exhibited high encapsulation efficiency and sustained release properties, resulting in enhanced cytotoxicity against ovarian cancer cells compared to free BJO. Functionalization with

anti-Epidermal Growth Factor Receptor (EGFR) fragments further enhanced targeted delivery efficacy, reducing tumor burden and extending survival in mouse models. Overall, *in vivo* studies using an intravaginal route could exploit these advantages, offering localized delivery directly to ovarian tumors via vaginal administration. This approach could potentially enhance drug retention, improve therapeutic outcomes, and reduce systemic exposure compared to traditional systemic administration routes. Further research in animal models and clinical trials would be essential to validate these findings and explore the full therapeutic potential of spongosomes in intravaginal drug delivery.

The analyzed studies demonstrate that non-lamellar lipid nanosystems can enhanced therapeutic efficacy across different routes of administration. These systems not only allow better drug penetration but also improve stability and bioavailability, optimizing therapeutic outcomes. Their unique nanostructures enable controlled drug release and targeted delivery, which are essential for successful therapy. Additionally, the versatility of these systems allows for the encapsulation of diverse therapeutic agents, including small molecules, peptides, and nucleic acids.

Non-lamellar lipid nanosystems demonstrate significant potential for improving cutaneous, ocular, and vaginal drug delivery. Future research should focus on optimizing these systems for various drugs and conditions and conducting larger-scale clinical trials to confirm their efficacy and safety.

Whereas, some studies present limitations, such as small sample sizes and limited observation periods. Additionally, the complexity of these systems may provide challenges in large-scale production and regulatory approval. Ensuring the reproducibility and scalability of non-lamellar lipid nanosystems is critical for their successful translation into clinical practice. Addressing these challenges requires collaboration between researchers, industry, and regulatory institutions.

These findings underscore the need for further research to optimize and customize non-lamellar lipid nanosystems for specific therapeutic applications. Future studies should focus on exploring new drug candidates, conducting extensive clinical trials, enhancing formulation stability and elucidating the mechanisms governing drug release and skin/ocular/vaginal penetration. In conclusion, non-lamellar lipid nanosystems constitute a cutting-edge strategy to topical drug administration, with considerable advantages over

traditional approaches and enormous potential for improving treatment results in a variety of clinical diseases.

Conclusion

The investigation into non-lamellar lipid nanosystems, which include cubosomes, hexosomes, and spongosomes, has paved the way for new advancements in drug delivery. These innovative systems have unique advantages over traditional lamellar nanosystems, such as enhanced drug solubility, stability, loaded high capacity, and controlled release profiles. Our comprehensive review revealed that these systems can have significant impact in three topical delivery routes: skin, ocular, and vaginal.

When it comes to topical drug delivery, these nanosystems enhance skin penetration and provide sustained drug release, making them valuable for treating infections, inflammation, and burns. These findings underscore the potential of these systems for dermatological applications.

Similarly, in ocular drug delivery, non-lamellar lipid nanosystems significantly improve drug bioavailability and therapeutic efficacy in ocular treatments. Their structural characteristics enable them to bypass ocular barriers, achieving effective drug penetration.

Likewise, for vaginal drug delivery, although less explored, vaginal administration of non-lamellar lipid nanosystems offers a promising approach for localized treatment of infections and other gynecological conditions. Initial studies suggest that these systems can provide controlled and targeted drug release, enhancing therapeutic effectiveness.

The studies reviewed reveal that non-lamellar lipid nanosystems significantly enhance drug delivery by improving penetration, stability, and bioavailability. These systems are versatile and may encapsulate a wide range of therapeutic agents, including small molecules, peptides, and nucleic acids. However, challenges remain in terms of scalability, reproducibility, and regulatory approval. Addressing these limitations requires interdisciplinary collaboration and the development of standardized protocols.

The potential of non-lamellar lipid nanosystems to revolutionize drug delivery is evident from the promising results observed in preclinical and clinical studies. Their ability to provide controlled and targeted delivery can lead to better therapeutic outcomes, reduced side effects, and improved patient compliance. The translation of these systems into clinical practice necessitates rigorous clinical trials to confirm their safety and efficacy.

Moreover, exploring their applications beyond topical, ocular, and vaginal delivery can further broaden their impact.

Consequently, future research should focus on optimizing the formulation and manufacturing processes of non-lamellar lipid nanosystems to ensure their scalability and cost-effectiveness. Larger-scale clinical trials are essential to validate their therapeutic potential and address any long-term safety concerns. Additionally, the exploration of new applications, such as oral and parenteral delivery, can expand the utility of these systems in various therapeutic areas. Collaborative efforts among researchers, industry stakeholders, and regulatory bodies are crucial for overcoming regulatory hurdles and accelerating the clinical adoption of non-lamellar lipid nanosystems.

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