Chapter

Breaking down the Barrier: Topical Liposomes as Nanocarriers for Drug Delivery into the Posterior Segment of the Eyeball

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Abstract

Topical instillation is the most widely preferred noninvasive route of drug administration to treat diseases affecting the anterior segment of the eye. Nonetheless, the ocular bioavailability for deeper ocular tissues is very low. Different routes of administration, such as intravitreal injections, periocular injections, and systemic administration, have been used to deliver drugs into the posterior segment ocular tissues. However, the presence of blood-retinal barriers (BRBs) makes systemic administration an impractical approach, whereas the drug delivery with the periocular administration route is compromised by ocular static and dynamic barriers. On the other hand, intravitreal injection, the most common and widely recommended route for drug administration to treat posterior ocular diseases, is related to several side effects such as endophthalmitis, hemorrhage, retinal detachment, and poor patient tolerance. Diverse strategies to overcome ocular barriers have been explored for topical drop formulations in order to deliver drugs into the posterior segment ocular tissues. In this chapter, we will review the promising topical nanocarriers for drug delivery into the posterior segment of the eye, emphasizing the use of liposomes for topical ophthalmic formulations targeting the vitreous cavity and the retina.

Keywords: liposomes, retina, vitreous, ocular posterior segment, nanocarriers, novel drug delivery system

1. Introduction and objective

The eyeball may be divided into two parts: the anterior and the posterior segments. The anterior segment consists of the pupil, cornea, iris, ciliary body, aqueous humor, and lens, whereas the posterior segment is comprised of the vitreous humor, macula, retina, choroid, and optic nerve. The retina is the sensitive layer which creates nerve impulses that are transmitted through the optic nerve to the brain. The macula is a specialized area of the retina that is responsible for the central, high-resolution color vision [1]. The retina and macula can suffer from

different disabling illnesses, such as age-related macular degeneration, cystoid macular edema, diabetic retinopathy, and ocular vascular occlusion, which are the leading causes of nonreversible vision impairment [2, 3]. These retinal disorders could be pharmacologically addressed; however, the efficient and safe delivery of drugs to the retinal tissue is not a completely solved subject.

Compared to drug delivery to other organs, ocular drug delivery faces significant challenges posed by various sophisticated ocular barriers. Many of these barriers are inherent and unique to ocular anatomy and physiology, making it a challenging task for drug delivery innovations. Corneal and conjunctival epithelium, blood-aqueous barriers (BAB), and blood-retinal barriers (BRBs) are the fundamental structures that restrict the passage of molecules and fluids to the retina and impede drug penetration; moreover, various elimination mechanisms, such as tear turnover, nasolacrimal drainage, protein binding, systemic absorption, and enzymatic degradation, limit the ocular bioavailability of drugs [4, 5]. Conventional drug administration systems such as eye drops, suspensions, and ointments are optimal in the treatment of the ocular surface (corneal and conjunctiva tissues) and anterior segment disorders. Nevertheless, due to the ocular barriers, drugs barely get into the posterior ocular segment [1]. It is well known that ocular bioavailability after the topical administration of a drug is generally <5% [4, 5].

Therefore, intravitreal (IVT) injections, which circumvent the ocular barriers, are the most frequent pathway to deliver drugs for the treatment of posterior ocular globe disorders. Today, the use of IVT injections has become the most common intraocular procedure worldwide [6] and the standard drug delivery method for the treatment of retinal diseases [2, 3]. Intravitreal injections are now routinely used for the intraocular administration of drugs such as corticosteroids, antimetabolites, antibiotics, and anti-VEGF therapies [7–11].

Although IVT injections are a well-described and feasible route for releasing drugs into the posterior pole of the eye, this procedure is associated with severe complications such as endophthalmitis, lens injury, and retinal detachment [12–14]. Moreover, it might be a burden for physicians, the health system, and patients with poor compliance in many cases [15]. Furthermore, the administration of IVT injections requires highly specialized human resources and special infrastructure, resulting in an expensive therapy option [16]. Research initiatives are continuously being proposed worldwide at fast pace by apex organizations and pharmaceutical companies in order to find a safer and more effective ocular drug delivery method for ocular use.

Nanostructured carriers or nanocarriers (nanomaterials) have proven to be an effective and slightly invasive drug delivery system to keep drug concentrations in the posterior segment of the eyeball, preventing the use of IVT injections or reducing their frequency. The advantage of using nanocarriers is their ability to increase the biopharmaceutical properties of the incorporated drug: solubility, stability, permeability, and retention at the site of application [17].

Nanocarriers are made of nanoparticles (NPs) (1–1000 nm), and they exemplify one of the multiple strategies of nanomedicine, which is interpreted as the application of NPs for medical purposes [18]. The most commonly used materials for NPs include lipids (liposomes), proteins (albumin NPs), cyclic oligosaccharides (cyclodextrins), synthetic polymers (polymeric micelles, dendrimers, hydrogel), and even inorganic compounds (cerium oxide NPs) [19]. Liposomes have been of much interest as carriers for advanced drug delivery in medicine and, especially, in ophthalmology for their potential to avoid the sophisticated ocular barriers, even when they are topically applied. In fact, liposomes and cyclodextrins are the only topically administered nanoparticles that have successfully proven to release corticosteroids in clinical assays [20, 21].

In the following sections, we will review the ocular barriers and routes for ocular drug delivery and discuss the different nanocarriers topically used in preclinical

studies that have proven to elude the anatomic and physiologic conditions that prevent drugs from reaching the tissues in the posterior segment of the eyeball, emphasizing the leading role of liposomes.

2. Barriers for ocular drug delivery

The different barriers that hinder intraocular drug delivery may be classified into three categories (**Table 1**).

2.1 Static barriers

The static barriers in the eye are physical barriers that prevent the diffusion of drug molecules into the posterior segment of the eye and the retina. The cornea is a mechanical barrier that comprises the anterior sixth of the eye. This transparent, ellipsoid barrier has different layers that play an important role in drug permeation: each layer offers a different polarity and a potential rate-limiting structure for drug permeation. The corneal epithelium is lipoidal in nature, and it contains 90% of cells in the cornea. These cells are joined by desmosomes and surrounded by ribbon-like tight junctional complexes. The presence of the *zonula occludens* delays paracellular drug permeation from the tear film into intracellular spaces of the epithelium, as well as into the inner layers of the cornea [22]. The epithelium has a significant resistance against the permeation of topical hydrophilic drugs. The highly hydrated stroma that comprises 90% of the corneal layer is made up by an extracellular matrix and a lamellar arrangement of collagen fibrils, and it is an important barrier against the permeation of lipophilic drug molecules. The hexagonal-shaped cells, or endothelium, represent the innermost layer of the cornea, and they have a selective

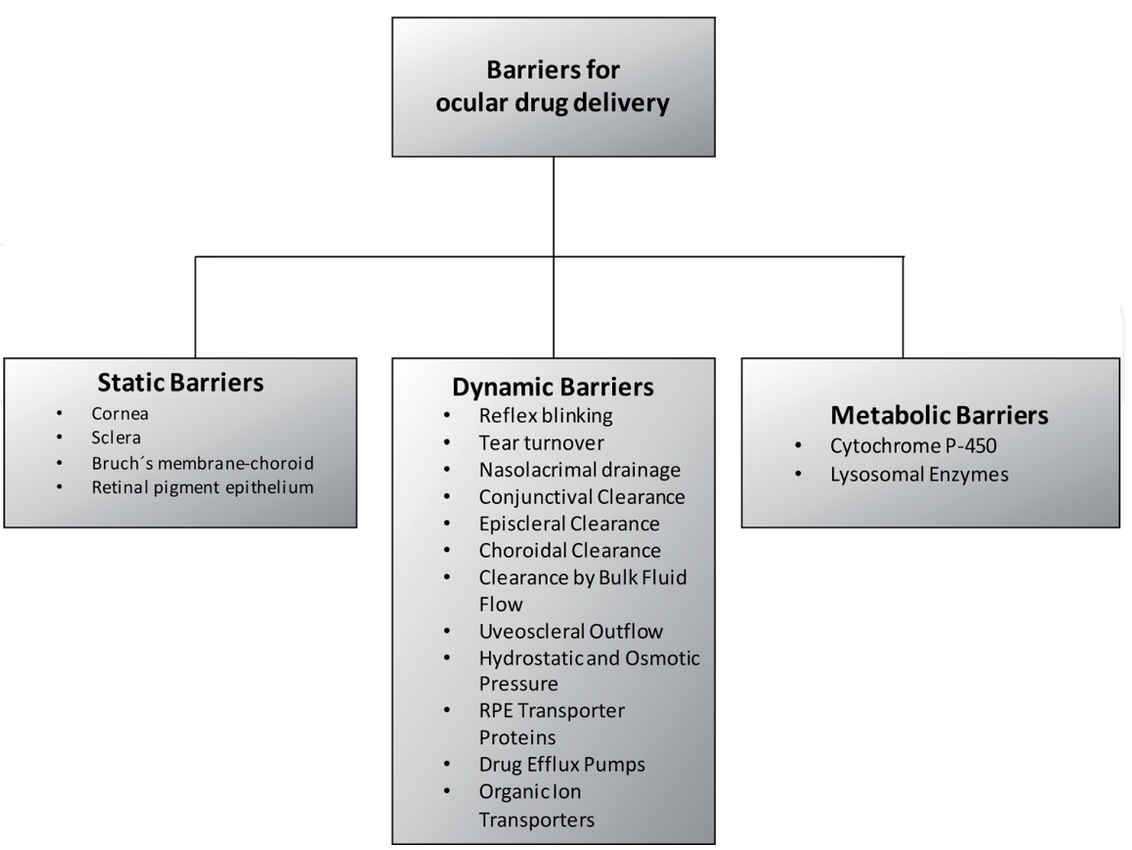

Table 1.
Types of barriers for ocular drug delivery.

carrier-mediated transport and a secretory function. New drug delivery systems should have an amphipathic nature in order to permeate through the cornea [23].

The sclera forms the firm, fibrous, outermost layer of the eye. It keeps the shape of the eye and provides an attachment for the insertion of the extraocular muscles. It is about 1 mm thick at the site where the optic nerve pierces it. The sclera is made of collagen and elastin chains that create a fiber matrix, where the pore diameter and intracellular spaces may determine the flow of drugs. Furthermore, the lateral orientation of fibers, the differences in the collagen architecture of the posterior sclera, and the differences in myopic eyes may affect drug transport with a lower or higher permeability depending on these changes. The permeability of the sclera for a number of molecules, such as dextrans, polyethylene glycol, anti-angiogenic molecules, antibiotics, oligonucleotides, and lipophilic compounds, has been measured [24–30]. There are different factors that may affect the scleral permeability of these molecules [2, 31–33]. Scleral permeability has a strong dependence on molecular weight, with smaller molecules having a better permeability. Similarly, since globular proteins are more permeable than linear dextrans of the same molecular weight, molecular radius is an important predictor of scleral permeability [32]. Finally, any surgical, pathological, or traumatic change in the anatomy of the sclera may lead to permeability changes [34].

Different studies on Bruch's membrane—the choroid and the retinal pigment epithelium (RPE)—show that permeability increases when lipophilicity does the same. Molecules that are passively transported across the RPE show similar permeability values in both outward (retina-choroid) and inward (choroid-retina) directions, while molecules that are actively transported show differences in permeability between the two of them [22]. The presence of RPE melanin may alter ocular drug disposition. Melanin binds to free radicals and drugs through electrostatic and van der Waals forces or through simple charge-transfer interactions with this pigment, which may alter the availability of the free drug at the targeted site [35]. All basic and lipophilic drugs bind to melanin; thereby, melanin binding may significantly lower pharmacological activity [36]. As a result of the presence of melanin, the binding of lipophilic compounds to the choroid-Bruch's membrane is higher; consequently, there is greater resistance to solute permeation across the choroid-Bruch's membrane than across the sclera, which is devoid of melanin [37–39].

Aging does not alter the permeability or ultrastructure of the sclera, but the permeability of Bruch's membrane and the choroid has shown to be significantly affected by age [2, 34]. Bruch's membrane may be a major resistance barrier against the movement of small solutes due to an increase in its thickness with aging (from 2 μm in the first decade of life to 4.7 μm in the tenth decade). Moreover, the accumulation of lipid-rich membranous debris and basal laminar deposits may have an important role in drug delivery [39].

2.2 Dynamic barriers

Dynamic barriers include the clearance through lymphatic and blood vessels, bulk fluid flow, and the active transport mechanisms of RPE transporter proteins. Precorneal barriers are highly effective and include solution drainage, blinking, tear film, tear turnover, and induced lacrimation.

Blinking is a normal reflex that protects the eye from dryness, bright light, and fingers or other objects coming toward it. Blinking also regulates tears, which nourish and cleanse the surface of the eye. The blinking rate in newborns is only two times per minute. This increases to 14–17 times per minute in adolescence and remains at this rate throughout the rest of the lifetime. Blinking may also increase in response to pain, bright light, changes in temperature and humidity, and conversations.

The tear film offers resistance, thanks to its high turnover rate. Mucin plays a protective role in tear film by forming a hydrophilic layer that moves over to the glycocalyx of the ocular surface and clears debris and pathogens [29, 40]. Human tear volume is estimated to be 7–10 μl, and the cul-de-sac can transiently contain around 30 μl of the administered eye drop. However, tear film displays a rapid restoration period of 2–3 min, and most of the topically administered solutions are washed away within just 15–30 s after instillation. Considering all of the precorneal factors, contact time with the absorptive membranes is lower, which is considered to be the primary reason for less than 5% of the applied dose reaching the intraocular tissues [41]. Precorneal fluid drainage is one of the main reasons for low ocular drug absorption [42–45].

After instillation, a big portion of an instilled volume (approximately from 80 to 90%) is drained into the nasolacrimal duct. Nasolacrimal drainage helps maintain the volume of precorneal fluid at about 7–10 μl at all times [46]. A natural protective physiological mechanism causes the loss of any excess fluid present: it is drained out through the nasolacrimal duct. Similarly, other factors such as the instilled volume, viscosity, pH, tonicity, and drug type may also alter the regular ocular physiological process. The higher the instilled volume, the higher the rate of solution drainage; increasing the drug's viscosity may extend contact time. Excessive tear production is associated with the instillation of acidic or alkaline solutions; hence the ideal ophthalmic formulation for topical delivery should be isotonic with tear secretions.

The conjunctiva is another effective barrier against ocular drug delivery. It is well vascularized, and drug molecules present in the conjunctiva and episcleral tissues are cleared through blood and/or lymphatic vessels [47, 48]. Subconjunctively injected tracers have been detected in the cervical lymph nodes within 6 min [49, 50]. Furthermore, molecular size and molecular radius may affect the rate of clearance [2]. Future drug delivery systems will have to consider conjunctival/episcleral clearance mechanisms, given that they play a significant role in reducing intraocular drug penetration.

The choroid is a dark brown, highly vascularized layer located between the sclera and the retina. It extends from the *ora serrata* to the aperture of the optic nerve in the sclera. Drug molecules that are topically, systemically, and orally administered may be eliminated by the uptake of the rapid blood flow of the choroid. Drugs can be carried away by bulk fluid flows in ocular tissues and are ultimately mostly cleared through choroidal vasculature or conjunctival vascular and/or lymphatic vessels. Uveoscleral drainage has been reported within a range of 4–60% [51], and it generates an outward bulk flow fluid from the suprachoroidal space. Many drugs may be carried away by the convective current of aqueous humor and cleared by the conjunctival vascular and/or lymphatic vessels. The effect of aging on uveoscleral outflow must also be considered by future drug delivery systems.

The osmotic pressure of the choroid in rabbits is 12–14 mmHg, and the vitreous humor has an osmotic pressure of approximately 0–1 mmHg [52]. This difference in osmotic pressure between the choroid and the vitreous generates a fluid flow toward the choroid. The hydrostatic pressure difference between the suprachoroid and the episcleral tissue also contributes to the outward bulk fluid flow [53]. There is a hydrostatic pressure difference of about 12 mmHg between the suprachoroid and the episcleral tissue, which works as a driving force for the outward bulk fluid flow. Drug efflux pumps, such as P-glycoproteins (P-gp) and multidrug resistance-associated proteins (MRPs), have been reported in RPE with an efflux effect directed toward the choroid [54, 55]. Finally, organic ion transporters may have an

important role as active transport for drugs depending on extracellular pH, temperature, and drug concentration [56].

2.3 Metabolic barriers

Transporters are membrane-bound proteins that play an important role in the active transport of nutrients or xenobiotics. These transporters play a significant role in humans in the processes of drug absorption, distribution, metabolism, and elimination. The presence of both efflux and influx transporters has been reported in various ocular tissues. The presence of efflux pumps in various ocular tissues has shown to regulate the intracellular drug concentration needed to achieve therapeutic activity. Drug-metabolizing enzymes are present in many ocular tissues such as the ciliary body and RPE [57, 58]. As a defense mechanism, the eye possesses several sophisticated metabolizing enzyme systems that degrade drug molecules, including those of the cytochrome P-450 (CYP) family, which are the most important drug-metabolizing enzymes. CYP3A isozyme, for example, metabolizes over 50% of commercial drugs [59].

3. Routes for drug delivery into the posterior segment of the eye

Designing a drug delivery system to target a specific tissue inside the eye is a challenge due to the different ocular barriers. Different modes of administration for drugs are available for ophthalmologists, but many of them fail to reach a therapeutic dose of the drug to elicit clinical effects. The different routes of ocular drug administration (**Figure 1**) include topical, oral/systemic, subconjunctival, subtenon, retrobulbar, intracameral, and intravitreal injections.

3.1 Topical administration

Topical administration is the most common method for drug administration in ophthalmology, mostly in the form of eye drops due to the ease of administration and the low cost. Topical application is useful in the treatment of disorders affecting the anterior segment of the eye [60]. The site of contact is the cornea, the conjunctiva, and the sclera. A normal eyedropper delivers from 25 to 56 μL of topical formulation with an average volume of 39 μL. However, an eye can transiently hold up to 30 μL, and the rest is lost either through nasolacrimal drainage or reflex blinking (5–7 blinks/min), significantly decreasing the overall drug available for therapeutic action. Furthermore, after a drop is administered to the eye, a major fraction of the drug is lost through lacrimation, tear dilution, nasolacrimal drainage, and tear turnover following topical administration. Such precorneal losses result in very low ocular bioavailability. Typically, less than 5% of the total administered dose reaches the aqueous humor [22, 61]. Therefore, in order to maintain minimum inhibitory concentrations, the agents need to be frequently dosed, resulting in poor patient compliance or potential adverse events. Physical barriers such as the cornea and the sclera and dynamic barriers such as reflex blinking, tear turnover, nasolacrimal drainage, conjunctival clearance, episcleral clearance, choroidal clearance, and clearance through bulk fluid flow and uveoscleral outflow are some of the many sophisticated barriers that cause poor drug molecule concentrations in the target organ (retina). For these reasons, topical administration is regarded as a poor option for the treatment of posterior diseases such as macular edema, age-related macular degeneration, and diabetic retinopathy.

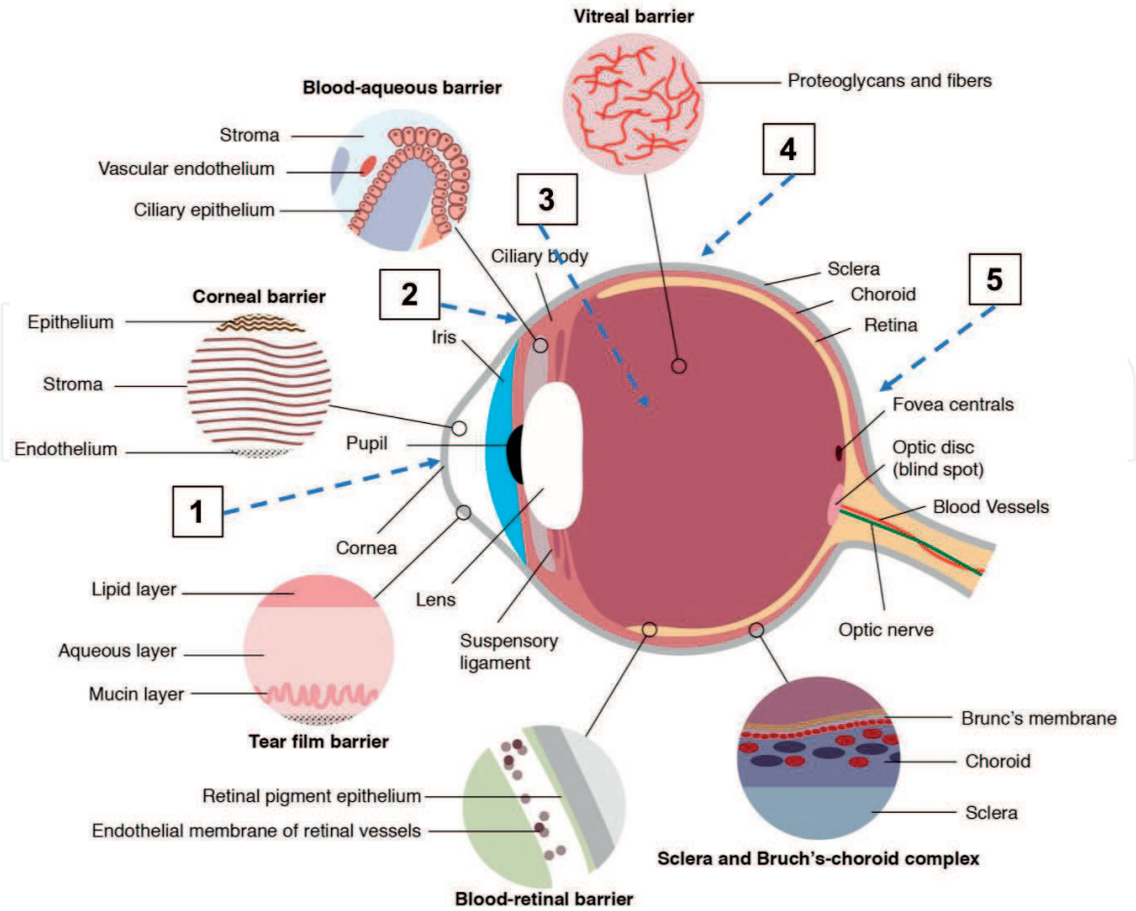

Figure 1.
Barriers for ocular drug delivery and different routes of drug administration to the eye. (1) Topical; (2) subconjunctival/subtenon; (3) intravitreal; (4) peribulbar; and (5) retrobulbar.

3.2 Oral/systemic administration

The oral and systemic administration of drugs is the most common drug delivery form in medicine (but this is not the case for eyes). Following oral/systemic administration, the blood-aqueous and blood-retinal barriers are major problems for drug delivery. The ciliary epithelium produces aqueous humor, which acts as an ultrafilter and restricts the entry of macromolecules such as antibiotics and plasma proteins [42]. Drug delivery to the posterior pole and the retina is very difficult due to the fact that blood-retinal barriers prevent the diffusion of drugs into the posterior pole of the eye. BRBs are composed of retinal capillary endothelial cells and RPE, which are known as the inner and outer blood-retinal barriers, respectively. RPE is a monolayer of highly specialized cells, and it aids in biochemical functions by selectively transporting molecules between photoreceptors and choriocapillaris [38]. However, the tight junctions of RPE efficiently restrict intercellular permeation.

Drugs can easily enter the choroid, thanks to its high vasculature compared to that of retinal capillaries. The choriocapillaris are fenestrated, resulting in rapid equilibration of drug molecules in the bloodstream with the extravascular space of the choroid. However, RPE restricts further entry of drugs from the choroid to the retina. Because the blood-retinal barrier, which is selectively permeable to more lipophilic molecules, mainly governs the entry of drug molecules into the posterior segment of the eye, this results in the need for frequent administration of high amounts of drugs, which leads to systemic side effects [62].

Although topical and systemic routes are convenient, the lack of adequate bioavailability and the failure to deliver therapeutic amounts of drugs into the retina prompted vision scientists to search for alternative routes of administration. Hence, specific oral or intravenous targeting systems are needed to transport molecules through the choroid and into deeper layers of the retina [43]. An example of a systemic drug for the treatment of retinal diseases is Visudyne, which is used in photodynamic therapy for the treatment of wet age-related macular degeneration (AMD) and central serous chorioretinopathy (CSC). However, owing to toxicity and delivery concerns, intravenous administration is not very common in treating ocular diseases.

3.3 Periocular administration

Although not very patient compliant, these routes are partly used to overcome the inefficiency of topical and systemic dosing in order to deliver therapeutic drug concentrations to the posterior segment of the eye globe. Moreover, systemic administration may lead to side effects, making it a less desirable delivery route for geriatric patients. Periocular routes include subconjunctival, subtenon, retrobulbar, and peribulbar administrations, and they are comparatively less invasive than intravitreal routes. Drugs administered by periocular injections can reach the posterior segment through three different pathways: the transscleral pathway, systemic circulation through the choroid, and the anterior pathway through the tear film, cornea, aqueous humor, and vitreous humor [63]. The administration of a drug via subtenon injection resulted in the highest and more sustained vitreous concentration of drug molecules compared to other periocular injections [64]. However, anterior segment complications have been described in some patients following periocular injections. The complications include ocular hypertension, cataract, hyphema, strabismus, and corneal decompensation [65]. Additionally, the concentration of many drugs, such as that of anti-angiogenic drugs, is better if an intravitreal injection is used as a delivery pathway.

3.4 Intravitreal injection

Nowadays, intravitreal injection is the preferred route for ocular delivery for the treatment of diseases of the retina. It offers important advantages over periocular injections as drug molecules are directly inserted into the vitreous chamber. This method involves the injection of a drug solution directly into the vitreous, via the pars plana, using a 30-Ga needle. Unlike other routes, intravitreal injection offers higher drug concentrations in the vitreous and the retina. The advantage of IVT injection is the circumvention of the blood-retinal barrier, which keeps most drugs out of the eye in the case of oral and systemic administrations [43]. Therefore, IVT administration is able to maximize the intraocular level of drugs in the vitreous and the retina while avoiding toxicities associated with systemic treatment [66–69]. The benefits of intravitreal therapies in retinal diseases have been well documented, and the current standard of care is the injection of a bolus of anti-VEGF into the vitreous cavity on a frequent basis. Such a large bolus of a drug has been associated with some side effects [70]. In addition, this administration procedure creates a typical curve with a large peak of drug concentrations and a rapid decay. Literature supports the fact that the half-life of anti-VEGFs, such as ranibizumab, is indeed very short (half-life = 2.6–4.0 days) [71], making it necessary to use a higher initial dose in order to exceed therapeutic levels so as to allow a longer 28-day treatment interval. Moreover, mathematical modeling demonstrates that the binding activity of 0.5 mg of ranibizumab is fivefold higher if given every 14 days instead of every 28 days [72].

Intravitreal injection can avoid ocular barriers for direct drug placement in the vitreous cavity, but drug distribution in the vitreous is not uniform. Small molecules can rapidly be distributed throughout the vitreous, whereas the diffusion of larger molecules is restricted. This distribution also depends on the presence of physiopathological conditions of the vitreous (vitrectomized patients present poor concentrations after 3 days) and the molecular weight of the administered drug [69]. In order to reach and maintain effective therapeutic concentrations, repeated injections are necessary. The frequent administration of drugs via this route can lead to endophthalmitis, damage to the lens, retinal detachment, and hemorrhage. Moreover, high acute intraocular drug concentrations may induce severe local toxicity and increase intraocular pressure. Additionally, it may be a burden for physicians, the health system, and patients with poor compliance in many cases [15]. Furthermore, IVT injections require highly specialized human resources and special infrastructure to apply them, and it is a costly option in developing countries [16].

Due to these limitations, new drug delivery systems are needed in order to find a safer and more effective ocular drug delivery method for the treatment of retinal diseases such as those from intraocular implants. Various drug delivery systems have been proposed using a group of biodegradable and nonbiodegradable platforms to passively deliver anti-angiogenic drugs [73–81]. However, one of the limitations of these drug delivery platforms is that, once implanted, they elute an unchangeable dosage of the drug and can only be stopped by explanting the device. In this context, they also share many of the problems of IVT injections.

4. Topical nanocarriers for drug delivery into the posterior segment of the eyeball

To date, different nanocarriers have proven to be capable technologies to circumvent most of the ocular barriers and effectively deliver drugs to the posterior segment of the eye. Their favorable physicochemical properties, such as the small size and adjustable surface, render them advantageous in targeting drugs to the retina. Although different administration routes have been analyzed, we consider that the topical route is the one of greatest interest because of its favorable characteristics such as its ease for the patient, a potentially better compliance, and lower incidence of serious complications than injected methods. The main topically proven nanocarriers are polymeric NPs, lipid nanoparticles, micelles, dendrimers, and liposomes.

4.1 Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are colloidal particles (10–1000 nm) where the drug is uniformly distributed throughout the particle matrix (nanospheres) or encapsulated inside a polymer shell (nanocapsules) [82]. Synthetic and natural biocompatible polymers can be used to develop PNPs. The polymers used to generate PNPs for ocular applications have been made from poly(alkyl cyanoacrylates) [83, 84], polycaprolactone, chitosan (CS), hyaluronic acid (HA), Eudragit (RS100 and RL100), Carbopol, gelatin, poly(butyl cyanoacrylate), polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA) [57, 85].

In regard to topical PNPs for posterior segment drug delivery, we would like to highlight the study performed by Tahara K et al., where submicron-sized poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were used to improve the efficiency of drug delivery to the retina through topically administered drugs. Chitosan and glycol chitosan (GCS), which are mucoadhesive polymers, and polysorbate 80

(P80) were used as surface modifiers of PGLA nanoparticles since this substance increases the interaction of NPs with cells. Coumarin-6 was used as a model drug and a fluorescent marker. The in vivo fluorescence image analysis showed detectable fluorescence intensity of coumarin-6 in the retina of mice after its topical administration [86]. The author proposed that the delivery of coumarin-6 into the posterior segment was associated with ocular surface modifications induced by CS, GCS, or P80. However, they did not verify this hypothesis.

Another interesting work concerning topically administered PNPs for retinal drug delivery was the study performed by Binstock et al., where the penetration of charged fluorescent nanoparticles into rabbit eyes using hydrogel iontophoresis was evaluated. Polyacrylic hydrogels were loaded with charged nanoparticles. NP suspensions (20–45 nm) were administered using cathodal and anodal iontophoresis and applying a current intensity of 1.5 mA for 5 min at the central cornea and the sclera (pars plana). The mean fluorescence intensity in outer ocular tissues (cornea, conjunctiva, and sclera) was 2–3 times higher for positively charged NPs with anodal iontophoresis. In deeper ocular tissues (retina, choroid, ciliary body, and iris), the mean fluorescence intensity was 5–15 times higher for the negatively charged NPs with cathodal iontophoresis. The positively charged particles demonstrated better penetration into inner ocular tissues than negatively charged particles [87]. This work showed that hydrogel iontophoresis combined with charged nanoparticles could be a potential technique for effective retinal drug delivery, but the investigation will have to be broadened and the potential adverse events explored.

4.2 Lipid nanoparticles

Three different types of lipid nanoparticles (LNPs) have been used for ophthalmic formulations: solid lipid nanoparticles (SLNPs), nanostructured lipid carriers (NLCs), and hybrid lipid nanoparticles. SLNPs are aqueous colloidal dispersions composed of solid lipids as a matrix material with the capacity to encapsulate hydrophilic and lipophilic drugs. SLNPs are provided with multiple advantages such as physical stability, protection of the encapsulated drug, controlled release, biocompatibility, feasible production, and the possibility of being sterilized by autoclaving [88–91]. On the other hand, SLNs have limited drug loading and expulsion for both hydrophilic and lipophilic drugs. Loaded drugs are usually located between fatty acid chains, in lipid layers, or in crystal imperfections [92]. NLCs consist of a mixture of solid and liquid lipids, and they exhibit a higher drug-loading capacity and longer stability than SLNPs [93]. Finally, hybrid lipid nanoparticles modified by multifunctional polymers [94] combine the merits of polymeric nanoparticles and lipid-based systems, which improve the pharmacokinetics and biodistribution of the loaded drug [95].

Experimentally, LNPs have been used for the delivery of substances to the posterior segment of the eye via corneal and non-corneal pathways. Araujo et al. [96] developed a nanometric (~200 nm), unimodal, and negatively charged NLC loaded with the fluorescent lipid, Nile red (NR-NLC) marker and a drug, triamcinolone acetonide (TA). The method used for the construction of the NLC was high-pressure homogenization. After NR-NLC eyedrop instillation in mice, lipophilic actives were released into the posterior segment of the eye. Retinal fluorescence of Nile red gradually increased over time, peaking 40 min after administration and almost disappearing at 160 min. The authors suggested that the fluorescence component of NR-NLC reached the retina through the BRB via non-corneal routes (sclera and conjunctiva).

In the work of Balguri et al., SLNPs and NLCs loaded with indomethacin (IN) were prepared through a hot homogenization method. Additionally, CS was used to modify the surface of SLNPs in order to increase penetration. After the topical application of formulations in rabbits, the concentration of IN was evaluated in

ocular tissues by using high-performance liquid chromatography (HPLC)-UV method. In vivo bioavailability studies demonstrated that the IN of the formulations (IN-SLNPs, IN-NLC, and IN-CS-NLC) reached the posterior ocular tissues 2 h post-topical application. Moreover, the IN-NLC showed higher drug-loading capability, higher entrapment efficiency, less drug expulsion, and, especially, the highest distribution concentrations of IN in deeper ocular tissues (vitreous humor, choroid-retina, and sclera). In vitro corneal penetration studies showed that CS increased corneal penetration by twofold (IN-CS-NLC compound), possibly thanks to the enhanced interaction with the mucus of the eye, the increased cellular uptake, and even its internalization. On the basis of their results, the authors proposed that the main transport routes of IN-NLC to successfully deliver IN to the posterior segment are the corneal pathway and the conjunctival-scleral pathway [97].

Finally, SLNPs comprising Compritol® 888 ATO and a polyethylene glycol (PEG) core, into which KTZ is dissolved, were topically used in rabbits. These SLNPs were generated by using a hot high-pressure homogenization method. Ocular pharmacokinetic studies indicated 2.5-fold and 1.6-fold increases in the aqueous humor and the vitreous humor via the topical administration compared to KTZ suspensions, respectively. Additionally, SLNPs were safe for the ocular surfaces of the study rabbits, and cell viability assays showed nontoxicity [98].

4.3 Polymeric micelles

Micelles (10–100 nm) comprise amphiphilic block copolymers above the critical micellar concentration with a hydrophobic core and a hydrophilic shell [99]. The preparation process of colloidal dispersions consists of the exposition of self-assembly oriented amphiphilic molecules in order to form core-shell monomer nanomicelles in a special solvent. These nanodispersions solubilize hydrophobic drugs by entrapping them within a mixed micellar hydrophobic core, resulting in clear aqueous formulations [100]. Micelles prevent or minimize drug degradation, improve drug permeation, increase the solubility and stability of drugs, and improve ocular bioavailability [101, 102].

Lately, nanomicelles/mixed nanomicelles loaded with different drugs such as dexamethasone [103, 104], voclosporin [105], and rapamycin [106] have been designed to reach the posterior segment of the eye. Liaw et al. [107] also demonstrated that micelles could be effective for intraocular gene delivery. In this work, pDNA with the lacZ gene was coupled to poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) nonionic copolymeric micelles. Following topical administration, gene expression was detected around the rabbit iris, sclera, and conjunctiva, and it was also seen in the intraocular tissues of nude mice after topical application for 48 h. In another study, Cholkar et al. [108] optimized an aqueous micellar solution of isopropyl ester carrying the resolvin (RX-10045) prodrug. Ocular tissue distribution studies demonstrated significant drug concentrations in anterior ocular tissues. Moreover, RX-10008 (the active metabolite of RX-10045) was detected in the retina/choroid upon topical drop instillation in rabbits. Micelles appeared to follow the conjunctival-scleral pathway to reach the retina, and no evidence of ocular damage after multiple dosing of this micellar solution was observed [108].

4.4 Dendrimers

Dendrimers are highly branched treelike structures with a core and many side chain [109] moieties. The size of dendrimer nanoparticles is usually under 100 nm, and peripheral functional groups (neutral, negative, or positive) may suffer secondary surface modifications in order to improve their characteristics for

ophthalmic use [110]. Due to their enhanced aqueous solubility, a large variety of surface groups, and their nontoxic nature, polyamidoamine (PAMAM) dendrimers have been investigated the most in regard to ocular drug delivery [111, 112].

Yavuz et al. [113] generated various anionic dexamethasone (DEX)-PAMAM complexes and evaluated the delivery of DEX to the posterior segment following topical administration in rats. The ocular tissue distribution study in rabbit eyes showed that anionic DEX-PANAM dendrimers reach the highest concentration of DEX in the vitreous body, the retina-choroid, and the sclera compared to topical DEX suspension. In addition, in vitro studies demonstrated that dendrimers with peripheral carboxyl or hydroxyl functional groups rarely showed cytotoxicity. Transport study results showed that dendrimer complexation increases DEX transport across both corneal and scleral tissues. Additionally, dendrimers have proven their efficiency to release genetic material when topically administered [114].

4.5 Cyclodextrin nanoparticles

Cyclodextrins (CDs) are a family of natural cyclic oligosaccharides which are usually composed of no more than 20 a-D-glucopyranoside units via the covalent conjugation of α-1,4-glycosidic linkages. CDs are cyclic structures whose hydrophilic outer surface is enclosed by the lipophilic internal cavity of low polarity. The features of their special structure predetermined their ease to form inclusion complexes for poorly water-soluble chemicals through non-covalent conjugations (electrostatic interactions, van der Waals contributions, hydrogen bonding, and charge-transfer interaction) [115]. CDs are not toxic for human beings [116], and they can improve the aqueous solubility, stability, activity, and dispersion of ophthalmic drugs [117].

Different types of oligomeric CD molecules are currently being used to produce cyclodextrin-based eye drops. These molecules are the β-CD [7], the γ-CD [8], its CD derivatives (hydroxypropyl-beta-cyclodextrin (HP-β-CD)), and the randomly methylated beta-cyclodextrin (RM-β-CD) [118]. Econazole nitrate (EC) and CD/HP-β-CD were complexed to form different EC-CD inclusion complexes through coprecipitation in order to improve ocular bioavailability [119].

In the study of Loftsson et al. [120], inclusion complexes of dexamethasone and γ-CD were topically administered to rabbits. After 2 h of application, DEX reached its highest concentration in the vitreous and the retina. The same group performed a clinical trial to prove the efficiency of topical 1.5% dexamethasone γ-cyclodextrin nanoparticle eye drops (DexNP) compared to posteriorly the subtenon injection of triamcinolone acetonide in diabetic macular edema (DME) patients. Topical DexNP significantly improved visual acuity and decreased the macular thickness of patients with DME. The effect was similar to that of subtenon triamcinolone. However, a modest increase in intraocular pressure (IOP) was noted with DexNP, but IOP normalized after the discontinuation of the eye drops [21].

4.6 Liposomes

Liposomes were described for the first time by Alec Bangham in the 1960s at the Babraham Institute, University of Cambridge, and they consist of single or multiple concentric lipid bilayers encapsulating an aqueous compartment.

Liposomes as carriers for advanced drug delivery have been of much interest lately. They are biocompatible vesicles composed of a phospholipid bilayer with structural resemblance to the cell membrane that form small spheroids that are able to carry both hydrophilic and lipophilic drugs (**Figure 2**). Phospholipids combined with water immediately form a sphere as one end is water soluble, while the opposite end is water insoluble. Water-soluble medications added to water are

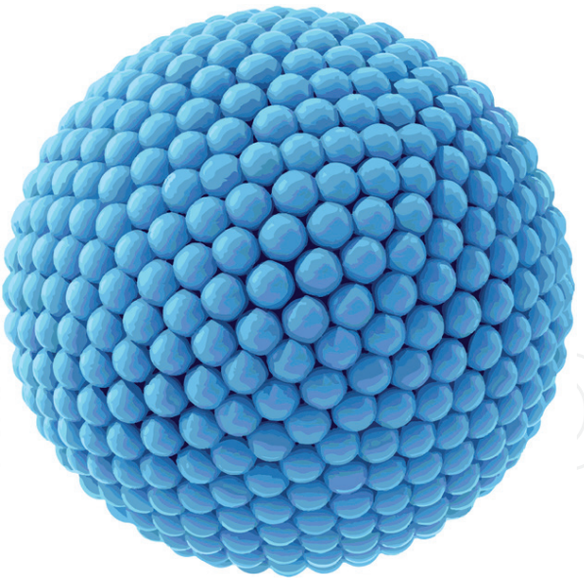

Figure 2.
Spherical formation of liposomes.

trapped inside the aggregation of hydrophobic ends and fat-soluble medications and incorporated into the phospholipid layer. Another significant characteristic of liposomes is their drug release rate, which depends on the liposomal composition, the nature of the drug, and the method of encapsulation.

Liposomes can be composed of naturally derived phospholipids with mixed lipid chains, like that of egg phosphatidylethanolamine, or pure components like dioleoylphosphatidylethanolamine (DOPE). The lipid bilayer can fuse with other bilayers (e.g., the cell membrane), thus delivering the liposomal contents. By including liposomes in solutions with DNA or drugs (which would normally be unable to diffuse through the membrane), they can be delivered past the lipid bilayer. The use of liposomes for the transformation or transfection of DNA into a host cell is known as lipofection. Liposomes can be created by sonicating phospholipids in water. Low shear rates create multilamellar liposomes, which have many layers, like an onion. Continued high-shear sonication tends to form smaller unilamellar liposomes.

4.6.1 Types of liposomes

Depending upon their structure, there are two types of liposomes, unilamellar liposomes or unilamellar vesicles, which have a single phospholipid bilayer sphere enclosing an aqueous solution (**Figure 3**), or multilayer liposomes, which are multilamellar structures (**Figure 4**). In multilayer liposomes, several unilamellar vesicles will form one inside the other in diminished sizes, creating a multilamellar structure of concentric phospholipid spheres (like a Matryoshka doll) separated by layers of water. The structural components of liposomes could be:

- Phospholipids such as phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, etc. For stable liposomes, saturated fatty acids are used.

- Sphingolipids like sphingomyelin.

- Sterols, such as cholesterol, with the potential to decrease the fluidity or microviscosity of the bilayer. It can reduce the permeability of the membrane to water-soluble molecules and stabilize the membrane in the presence of biological fluids such as plasma.

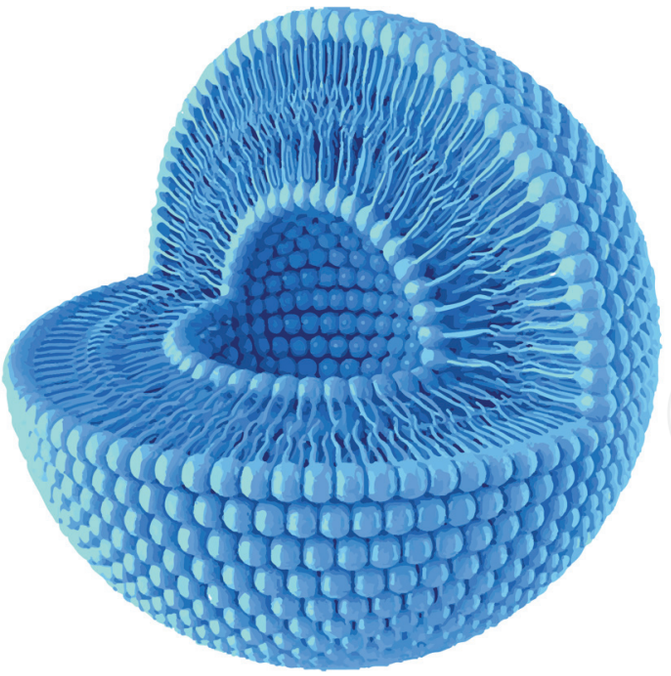

Figure 3.
Single-layer liposome.

Figure 4.
Multilayer liposome.

- Synthetic phospholipids (saturated and unsaturated).
- Polymeric materials. Polymerized liposomes have significantly higher permeability barriers to entrap aqueous drugs.
- Polymer-bearing lipids. Coating liposome surfaces with charged polymers results in repulsion interactions with macromolecules.
- Cationic lipids such as dioctadecyldimethylammonium bromide or chloride.

4.6.2 Size of liposomes

Due to differences in preparation methods and lipid compositions, liposomes may be classified, according to their size, in small (<100 nm), medium (100–250 nm), or large (>250 nm) [121, 122]. Some liposomes may be as small as <0.1 μm or as big as >1 mm in size.

4.6.3 Preparation method (incorporating drugs)

In addition to the ability to entrap drugs with different solubility characteristics, it has been hypothesized that liposomes have different release kinetics. In general, multilamellar liposomes are more easily formed at larger hydrodynamic diameters, and, therefore, they have greater entrapped volumes than unilamellar liposomes. As a result of this, unilamellar liposomes with a hydrodynamic diameter of 130 nm exhibit a much faster release rate than multilamellar liposomes with two to three lamellar bilayers and a hydrodynamic diameter of 250 nm [123, 124]. The difference in the release rate is overall due to the number of phospholipid bilayers that it has to cross before being released [123, 124]. The ongoing interest of researchers in liposomal characteristics such as their stability, pharmacokinetic properties, and therapeutic efficacy has led to second-generation liposomes through the modulation of their lipid composition, size, and the charge of the vesicle. The addition of cholesterol to the lipid bilayer of liposomes reduces their permeability, increases their in vivo and in vitro stability, and can be used to anchor other molecules such as polyethylene glycol (PEG) or deoxyribonucleic acid (DNA) to the liposomes for their application in biosensing or as "stealth" drug carriers [124]. The use of phosphatidylcholine with saturated fatty acyl chains and materials that stretch transition temperatures beyond 37°C offered even greater stabilization [115, 125]. Furthermore, hydrophilic carbohydrates or polymers, such as monosialoganglioside (GM1) and PEG, were included in the liposomal composition. GM1 can lead to the prolongation of the in vivo liposome viability time [126–129].

There are many different methods to prepare liposomes. According to Bozzuto and Molinari [130], the choice of the method depends on factors such as the physicochemical characteristics of liposomes and/or drug components; the toxicity and concentration of the loaded drug; the type of medium in which liposomes are dispersed; the additional process during the application/delivery of liposomes; the desired size for the application; the half-life desired for successful application; costs, reproducibility, and applicability regarding large-scale production for clinical purpose; and good manufacturing practice-relevant issues. In addition, the target organ is a significant issue to be considered when planning the preparation of liposomes.

Every method for preparing liposomes involves four basic stages: drying down lipids from an organic solvent, dispersing the lipid in aqueous media, purifying the resultant liposome, and analyzing the final product. The most common methods for producing liposomes, according to Gabizon et al. [123, 128] and Akbarzadeh et al., [131] are the following.

5. Mechanical dispersion method

5.1 Handshaking and non-handshaking method

In order to produce liposomes, lipid molecules must be introduced into an aqueous environment. When a dry lipid layer film is hydrated, lamellae swell and grow

into myelin figures. Mechanical agitation provided by vortexing, shaking, swirling, or pipetting causes myelin figures to break and reseal the exposed hydrophobic edges, resulting in the formation of liposomes, which can be made by using the handshaking method.

5.2 French pressure cell method

It involves the extrusion of multilamellar vesicles (MLV) through a small orifice. The method involves the gentle handling of unstable materials. The resulting liposomes are larger than those generated by using the sonicated method. However, high temperatures are difficult to obtain and working volumes are small.

5.3 Freeze-thawed liposome method

The creation of unilamellar vesicles is a result of a fusion of UV throughout the processes of rapidly freezing and slowly thawing. The encapsulation rates go from 20 to 30%.

5.4 The sonication method

The sonication method is the most used method for the preparation of liposomes (unilamellar). Both techniques are *probe sonication*, in which the tip sonicator is directly immersed into the liposome. Dispersion is high and there is overheating, so the vessel is submerged in an ice bath. With this technique, up to 5% of the lipids can be de-esterified after 1 h. The *bath sonicator* is the second type of sonication method, and the dispersion of liposomes in a tube is placed into a bath sonicator. The regulation of the temperature is easier, and the lipid bilayer of liposomes can fuse with other bilayers, thus delivering the liposomal contents. By making liposomes within drug solutions, they can be delivered past lipid bilayers.

6. Solvent dispersion method

6.1 Ether injection method

Ether injection method is also known as solvent vaporization. A solution of lipids dissolved in diethyl ether or an ether-methanol mixture is injected into an aqueous solution of the drug in order to be encapsulated at 55–65°C or under reduced pressures. The removal of ether under vacuum leads to the formation of liposomes.

6.2 Ethanol injection method

A lipid solution of ethanol is rapidly injected to a huge buffer excess. Multilamellar vesicles are at once formed. Unfortunately, the population is not homogeneous with this method.

6.3 Reverse-phase evaporation method

Reverse-phase evaporation method is based on the formation of inverted micelles. These inverted micelles are formed upon the sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes, and an organic phase, in which the amphiphilic molecules

are solubilized. The slow removal of organic solvent leads to the transformation of these inverted micelles into a gel-like, viscous state. After the gel-state collapses, some of the inverted micelles disintegrate, but the excess of phospholipids in the environment contributes to the formation of a complete bilayer around the remaining micelles, which results in the formation of liposomes.

7. Detergent removal method

7.1 Dialysis method

As the detergent is detached, the micelles increasingly become better-off in phospholipids, and, lastly, they combine to form UVs.

7.2 Detergent removal of mixed micelle method

The absorption method is attained by shaking mixed micelle solution with beaded organic polystyrene absorbers.

7.3 Gel permeation chromatography method

In this method, the detergent is depleted through size special chromatography.

7.4 Dilution method

The spontaneous transition from polydispersed micelles to vesicles occurs due to the dilution of the aqueous micellar solution of detergent and phospholipids with buffer.

7.5 Stealth liposomes and conventional liposomes

Liposomes become known by the mononuclear phagocytic system following contact with plasma proteins. This is solved through the use of synthetic phospholipids, particle coated with amphipathic polyethylene glycol, coating liposomes with chitin derivatives, freeze drying, polymerization, and microencapsulation of gangliosides. A stealth liposome is a sphere-shaped vesicle with a membrane that is composed of a phospholipid bilayer used to deliver drugs or genetic material to a cell.

Drug loading can be achieved through passive (if the drug is encapsulated during liposome formation) or active methods (after liposome formation). Freeze-dried (lyophilization) liposomes are formed from preformed liposomes at tremendously low pressures. Very high encapsulation efficiencies, even for macromolecules, can be achieved using this method. During dehydration, the lipid bilayers and the drug to be encapsulated into the liposomes are brought into close contact. Upon reswelling, the chances for the encapsulation of the adhered molecules are much higher. Rehydration is a very important step and it should be very carefully done. The aqueous phase should be added in very small portions with a micropipette to the dried materials. After each addition, the tube should be thoroughly vortexed.

7.5.1 Transportation mechanisms

Liposomes can interact with cells through different mechanisms [129]:

Endocytosis. Carried out by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils.

Adsorption to the cell surface either through nonspecific weak hydrophobic or electrostatic forces or specific interactions with cell surface components.

Fusion with the plasmatic cell membrane through the insertion of the lipid bilayer of the liposome into the plasma membrane with the simultaneous release of the liposomal content into the cytoplasm.

Transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of liposomal contents.

7.5.2 Evaluation

Liposomal formulation and processing for a specific purpose are characterized for ensuring their predictable in vitro and in vivo performance [132]. Characterization parameters for the purpose of evaluation may be classified into three broad categories that include:

Physical characterization. It evaluates the size, shape, surface features, lamellarity, phase behavior, and drug release profile.

Chemical characterization. It includes studies in order to establish the purity and potency of various lipophilic constituents.

Biological characterization. Biological characterization is helpful in establishing the safety and suitability of the formulation for therapeutic applications.

Some parameters are the vesicle's shape and lamellarity, vesicle size and size distribution, encapsulation efficiency (expressed as percentage (%)), phase response, and transitional behavior and drug release. Zeta potential (ZP) refers to the potential difference between the electric double layer (EDL) (an adsorbed double layer developed on the surface of dispersed charged particles) of movable particles and the layer of dispersant around them at the slipping plane. A stabilized nanosuspension is a suspension that may be affected by several factors such as pH, ionic strength, and the concentration of particles. The phospholipid composition of liposomes is the main content that influences the overall surface charge of liposomes.

7.5.3 Applications of liposomes in medicine

Liposomes are versatile carriers for the delivery of numerous challenging molecules, and they have remarkable advantages compared to other colloidal systems. They have been investigated for a wide range of applications in pharmaceutical technology through topical, transdermal, nasal, and oral routes for efficient and effective drug delivery. Liposome formulations have potential advantages that other drug delivery systems do not have:

i. Suitable for the delivery of hydrophobic, amphipathic, and hydrophilic drugs.

ii. They protect the encapsulated drug from the external environment.

iii. Reduced toxicity and increased stability.

iv. Increased efficacy and therapeutic index of the drug.

v. The sustained-release system of systematically or locally administered liposomes.

vi. Improved transfer of hydrophilic, charged molecules.

vii. Improved penetration into the tissues.

viii. Site avoidance mechanism.

ix. They offer site-specific targeting.

x. Nontoxic, flexible, biocompatible, and completely biodegradable.

xi. Size may vary in order to incorporate smaller or larger drug molecules.

xii. They can be administered through various routes.

xiii. The therapeutic activity of chemotherapeutic agents may be improved through liposomal encapsulation. This reduces deleterious effects similar to or lower than those required for maximum therapeutic activity.

xiv. They help reduce exposure of sensitive tissues to toxic drugs.

Some of the disadvantages of liposomes are:

i. Production cost is high.

ii. Leakage and fusion of encapsulated drug molecules with certain preparation methods.

iii. Short half-life.

iv. Less stability.

v. Low solubility.

vi. Phospholipids undergo oxidation and hydrolysis.

vii. Leakage and fusion.

viii. Allergic reactions to liposomal constituents may occur.

Liposomal formulations have several applications in cancer chemotherapy. Due to the nature and behavior of cancer tissues and the large difference between them and regular tissues, cancer tissues are considered an appropriate target for liposome drug delivery systems. For example, the tumor vasculature is characterized by a leaky vasculature and limited lymphatic drainage; consequently, drug molecules can easily be accumulated in intercellular spaces of a large variety of tumors. Numerous different liposome formulations of several anticancer agents were shown to be less toxic than free drugs [131, 133–135]. Examples of these drugs are doxorubicin and daunorubicin citrate for Kaposi sarcoma; doxorubicin for ovarian cancer and solid tumors; nystatin, vinorelbine, cisplatin and its analog docetaxel, tretinoin, siRNA, topotecan, irinotecan, paclitaxel, and camptothecin for solid tumors; cisplatin and its analog for colorectal neoplasms, Grb-2 in leukemia; Bcl-2 for lymphoma; BikDD for pancreatic cancer; and DOTAP (Chol-Fus I) for the

treatment of lung cancer [130]. Many other drugs are being researched in order to make them affordable for liposomal drug delivery systems.

In dermatology, liposomes have successfully been used in atopic dermatitis (taxifolin glycoside) as antibacterial, antifungal (metronidazole nitrate, amphotericin B), and anti-leishmaniasis treatments (amphotericin B, meglumine antimoniate). Antioxidants such as natural flavonoids (catechin and naringenin) have been used to prevent the oxidation of cutaneous disorders and as photoprotective (quercetin), antipsoriatic, anti-inflammatory agents and anti-acne drugs [136].

In the same context, prostaglandin has been used in peripheral vascular disease, meglumine antimoniate for cutaneous leishmaniasis, fentanyl for pain relief, amikacin in cystic fibrosis, and prilocaine in dental anesthesia, all of them are more examples of liposomal drug products for medical use [136]. Furthermore, antihypertensive drugs have been used for the management of cardiovascular disorders (propanol hydrochloride, valsartan, and nifedipine have been developed in liposomal formulations) [136]. There are publications regarding local anesthetic drugs [137–139], drug delivery to the brain in anti-migraine and anti-Parkinson drugs [140–143], and the nasal delivery of liposomal formulations (salbutamol).

The application of liposomes in vaccine formulations and toxoids is one of the main achievements of modern medicine. Vaccination activates particular parts of the immune system in order for it to express specific immune responses followed by the induction of long-lasting immunological memories to defend against subsequent infectious attacks [144]. Most available immunizations are intramuscularly delivered, which is painful and requires an aseptic technique, as well as skilled and trained personnel for their administration. Thus, biological products (vaccines and toxoids) are suitable as a noninvasive approach compared to conventional methods, and they have numerous obvious advantages such as increased patient compliance, reduced systemic side effects, and constant plasma concentrations. Ding et al. reported on antigens such as vaccines and toxoids. Depending on the type of antigens, the dose to be delivered, immunization schedule, the presence of co-stimulatory factors, and liposomal composition, the immunization of antigens loaded in ELs elicits an effective immune response with serum IgG levels comparable to those obtained after subcutaneous injections [145–148]. Transcutaneous immunization (TCI) is a novel technique, and it requires the simple introduction of antigens into the host tissue through topical application on the skin [148]. This offers ease of administration and the potential to elicit a robust immune response as compared to conventional painful (needle injections) methods prescribed in equivalent doses [145].

Image-guided delivery is another opportunity area for liposomal formulations. Imaging plays an integral part in modern precision and individualized medicine. Wide applications of imaging such as monitoring drug delivery, accurate diagnosis of diseases, determining the response to therapy, and guiding minimally invasive procedures are some of the applications of imaging in the clinic. However, traditional imaging modalities, such as computed tomography (CT), positron-emission tomography (PET), magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT), all suffer from target specificity, which limits their clinical utility. Nanoparticles, with their versatility in surface functionalization, provide opportunities to enhance target specificity and label NPs with various isotopes, which enables them to act as contrast agents. Recent developments in multimodality imaging to better diagnose diseases and monitor treatments have embarked on using liposomes as a diagnostic tool. Conjugating liposomes with different labeling probes enable the precise localization of these liposomal formulations by using various modalities such as PET, SPECT, and MRI [149].

Glucocorticoids (GC) are one of the most popular and versatile classes of drugs available to treat chronic inflammation and cancer, but side effects and resistance constrain their use. In order to overcome these hurdles, which are often related to the uniform tissue distribution of free GC and their short half-life in biological fluids, new delivery vehicles have been developed, including PEGylated liposomes, polymeric micelles, polymer-drug conjugates, inorganic scaffolds, and hybrid NPs. While each of these nanoformulations has individual drawbacks, they are often superior to free GC in many aspects, including therapeutic efficacy when tested in cell cultures or animal models. The characterization and pharmacokinetics of triamcinolone acetonide-loaded liposomal topical formulations for vitreoretinal drug delivery [150] have been published by Altamirano-Vallejo et al. They showed that a formulation with triamcinolone acetonide-loaded liposomes is feasible and that it has the potential to reach the vitreous cavity with a significant concentration of the drug.

Liposomal vesicles have potential advantages when compared to conventional drug delivery methods. Liposomes in different forms are still one of the most investigated drug delivery carrier systems for the ocular delivery of drugs in both preclinical studies and clinical trials. They seem to be an almost ideal drug-carrier system since their morphology is similar to that of cellular membranes and because of their ability to incorporate various substances. They are valued for their biological and technological advantages as optimal delivery systems for biologically active substances, both in vitro and in vivo, and are considered to be the most successful drug-carrier system known to date [151].

7.5.4 Liposomes in ophthalmology

Several liposome formulations have been used in ophthalmology to target the anterior segment of the eye. The use of liposomal formulations to enhance the bioavailability of topical applied acyclovir and ganciclovir has been evaluated with promising results [152]. Antibacterial drugs, such as tetracycline, gentamycin, ciprofloxacin, norfloxacin, and chloramphenicol, have been prepared as liposomal solutions with higher activity compared to that of standard solutions [153]. Antifungal agents such as amphotericin B and fluconazole have been under research [152], and liposomal formulations were highly effective in treating *Candida* keratitis. Anti-inflammatory drugs and immunomodulatory agents are widely used in the treatment of ocular inflammatory and immunological diseases. In order to enhance ocular bioavailability and reduce the toxic effects following topical or intravitreal administration, liposomal forms of many of these drugs have been evaluated (diclofenac, cyclosporine, tacrolimus) with promising results and better concentrations than standard formulations [152, 153]. Similarly, liposomal antiglaucoma agents' formulations (pilocarpine, latanoprost, acetazolamide) were more effective in reducing intraocular pressure (IOP). In the same context, lubricants and antioxidants have also other potential uses for liposomal formulations.

Liposomal drugs that have transitioned from preclinical research to clinical phase trials include latanoprost-loaded conventional liposomes developed for subconjunctival administration [154]. Phase I and II trials on the safety and efficacy of latanoprost-loaded liposomes in the treatment of ocular hypertension and primary open-angle glaucoma have been completed (ClinicalTrials.gov identifier, NCT0198732357; ClinicalTrials.gov identifier, NCT02466399).

7.5.5 Topical liposomes for drug delivery into the posterior segment of the eyeball

Different efforts have been performed to deliver drugs into the posterior segment of the eye through the instillation of loaded liposomes (**Table 2**) [155–163].

Drug	Liposome description	Liposomal composition	Synthesis method	Main findings	Clinical trials	Reference
Coumarin-6	Poly-L-lysine surface-modified liposomes	Poly-L-lysine, EPC, DCP, and CH	Hydration method	Noncytotoxic in corneal and conjunctival cells	No	[155]
Coumarin-6	Submicron-sized liposomes (ssLips)	DSPC, EPC, DCP or SA, and CH	Hydration method	Delivery efficiency of coumarin-6 to the retina was altered depending on particle size	No	[157]
Coumarin-6	Submicron-sized liposomes (ssLips)	EPC or DSCP, DCP, and CH	Hydration method	The magnitude of fluorescence in the retina was closely related to both liposome rigidity and particle size. Images of the entire eye showed that ssLip was delivered via the non-corneal pathway after administration	No	[162]
5(6)-Carboxyfluorescein	Submicron-sized liposomes (ssLips)	DSPC and EPC	Calcium acetate gradient method that implies hydration and is freeze-thawed	Luminescence intensity in the retina was higher when a ssLip formulation composed of DSPC was applied	No	[161]
Diclofenac	PVA 205 or PV-R surface-modified liposomes	PVA 205 or PV-R, DSPC, and CH	Calcium acetate gradient method that implies hydration and is freeze-thawed	The increase in particle size of the liposomal formulation was inhibited in the presence of PVA 205 or PV-R. In vivo animal study revealed that the concentration of diclofenac in the retina-choroid was enhanced 1.8-fold through surface-modified liposome entrapment compared to that of the unaltered diclofenac solution	No	[158]
Edaravone	Submicron-sized liposomes (ssLips)	EPC and CH	Calcium acetate gradient method that implies hydration and is freeze-thawed	Edaravone-loaded ssLips showed a stronger inhibition of in vitro light-induced ROS production and cell death than free edaravone. ssLips showed modest cytotoxicity	No	[159]
Triamcinolone acetonide	Triamcinolone acetonide-loaded liposomes formulations (TA-LF)	Polyethylene glycol (PEG-12) glyceryl dimyristate	QuSomes®; self-forming, thermodynamically stable	TA-LF, topically administered, can deliver TA to the vitreous cavity and efficiently reach the retina with no adverse effects in rabbits. TA-LF was well tolerated and improved the best corrected visual acuity and the central foveal thickness in patients with refractory pseudophakic cystoid macular edema	Yes	[150]

Drug	Liposome description	Liposomal composition	Synthesis method	Main findings	Clinical trials	Reference
Triamcinolone acetonide	Triamcinolone acetonide chitosan-coated liposomes (TA-CHL)	Chitosan, phosphatidylcholine, and CH	Calcium acetate gradient method that implies hydration and is freeze-thawed	TA-CHL penetrates into the posterior segment of the eye. Chitosan-coated liposomes were a more efficient ocular delivery system of triamcinolone acetonide to the posterior segment of the eye as eye drops than non-coated liposomes	No	[164]
Bevacizumab	Annexin A5-surface-modified liposomes	Phosphatidylserine, phosphatidylethanolamine, and anionic phospholipid-binding protein annexin A5	Calcium acetate gradient method that implies hydration and is freeze-thawed	Annexin A5-surface-modified liposomes deliver physiologically significant concentrations of bevacizumab to the posterior segment of rat eyes (127 ng/g) and rabbit retinas (18 ng/g). Annexin A5-mediated endocytosis enhances the delivery of bevacizumab	No	[156]
pDNA	pDNA/PEI complex-loaded liposomes modified with transferrin	EPC and CH	Detergent removal method	The modification of ligand (transferrin), which binds to a specific receptor in RPE cells to the liposomes, improves gene delivery efficacy to the posterior segment of the eye	No	[160]
Plasmid DNA with β-galactosidase gene	TMAG liposome and DC-CH liposome	TMAG liposomes, TMAG and DOPE; DC-CH liposome, DC-cholesterol and DOPE	Hydration method	Gene expression was found in retinal ganglion cell until 1 month after the topical application of liposomes	No	[163]

CH, cholesterol; DC, dicetyl phosphate; DC-cholesterol, 3beta [N-(N'-N'-dimethylaminoethane)-carbamyl] cholesterol; DLPC, dilauroylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DSPC, L-α-distearoyl phosphatidylcholine; EPC, egg phosphatidylcholine; PEI, polyethylenimine; PVA 205, polyvinyl alcohol; PVA-R, polyvinyl alcohol derivatives bearing a hydrophobic anchor (C16H33 S) at the terminal of the molecule; SA, stearyl amine; TMAG, N-(alpha-trimethyl ammoniacetyl)-didodecyl-D-glutamate.

Table 2.
Reported liposomes for drug delivery to the posterior segment of the eyeball.

For example, fluorescent probes used as drug models, such as coumarin-6 and 5(6)-carboxyfluorescein, have efficiently been released into the posterior segment of the eyeball by liposomes [155, 157, 161, 162]. On the other hand, drugs like edaravone and diclofenac were successfully released into the vitreous and the retina by liposomes [158, 159].

Special mention is reserved for the study performed by Davis BM et al. This group demonstrated that the topical instillation of eye drops containing annexin A5 associated with liposomes loaded with bevacizumab is able to deliver physiologically significant concentrations of this large therapeutic protein (monoclonal antibody against vascular endothelial growth factor A) into the posterior segment of the eye in animal models (rats and rabbits) [156]. Moreover, liposomes can release genetic material into the vitreous and the retina [160, 163].

Lastly, a topical triamcinolone acetonide-loaded liposomes formulation (TA-LF) was used to successfully deliver TA into the vitreous and the retina of rabbits. Besides the authors report that TA-LF was well tolerated by the study animals and that no toxicity was observed in cell culture assays and no adverse events like corneal and conjunctival erosions were observed [150]. Recently, Jin Li et al. [164] validated in animal models (mice) that eye drops containing chitosan-coated liposomes carrying TA are an efficient method to deliver this drug into the posterior segment of rabbit eyeballs, supporting the previous findings published by Altamirano-Vallejo et al. Even though Li et al. reached superior TA entrapment efficiency in their TA-loaded liposomes prepared through the calcium acetate gradient method, it seems that this characteristic does not compromise the therapeutic activity of TA-LF. TA-LF has been tested in clinical assays where its efficiency and safety profile have satisfactorily been demonstrated. In a recent report, TA-LF was efficient in the management of refractory pseudophakic cystoid macular edema [20], where the use of this formulation was associated with the improvement of best corrected visual acuity and central foveal thickness with no reports of adverse events. At this time, phase II trials are underway to demonstrate the efficacy of TA-LF for macular edema.

8. Conclusion

Although there has been great interest in the development of new topical ocular delivery systems, the topical administration of drugs is not as effective as intravitreal delivery in treating retinal diseases. During topical delivery, drug molecules must cross several anatomic and physiologic barriers before reaching the posterior segment of the eye. Consequently, very low concentrations, with almost no clinical effect, are usually obtained. However, dramatic changes have been observed in the field of ocular drug delivery over the last decade, and nanomedicine is one of the most promising technologies for efficient drug delivery into intraocular tissues. Nanocarriers have a great potential to solve the challenge of releasing drugs into the posterior segment of the eyeball and reaching deeper intraocular tissues. Different nanocarriers, such as NPs, LPs, micelles, dendrimers, cyclodextrin nanoparticles, and liposomes, have been developed for the safe and effective drug delivery to the vitreous and the retina. Nevertheless, only a few of these particles have clinically been approved (liposomes and CDs among them).

Liposomes have lately been of great interest as carriers for advanced drug delivery in medicine and, especially, in ophthalmology due to their potential to avoid sophisticated ocular barriers when they are topically used. Liposomal drug delivery systems have a bright future in ophthalmology and, particularly, for the retina for several reasons. First, features such as the passive-targeting effect may substantially

enhance the amount of drug in target tissues. Second, liposomal technology allows the therapeutic index of already established drugs, with well-established therapeutic profiles, to be improved. This eliminates some of the considerable risks associated with the development of new pharmaceutical products. Third, many of the potential new pharmaceutical products arising from advances in biotechnology would come from macromolecules such as proteins, peptides, oligonucleotides, and plasmids, which could easily be carried by liposomes so as to access target tissues and cells. Additionally, liposomes possess features of optimal drug delivery systems to get to the ocular surface and intraocular tissues, for instance, sustained and controlled drug delivery, ease to instill for the patient, good corneal-scleral penetration, and the fact that they reach appropriate concentrations of the drug in the target tissue, avoid side effects produced by conventional systems (like IVT injections), there is better patient compliance, and they are potentially affordable for patients. A proof of the clinical advantages of liposomes is represented by recent trials where TA-loaded liposomes have successfully been used to treat macular edema.

Considering that liposomes collect the main characteristics of an optimal drug delivery system and that these nanocarriers have proven their capacity not only to deliver drugs but also genetic materials to the posterior segment of the eye, we consider that liposomes are promising nanoparticles for the therapy of multiple intraocular illnesses.

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