Archives of Pharmaceutical Sciences Ain Shams University 2021; Vol. 5(1): 71-96

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Pharmaceutics

Review Article

Appraising the Competency of Lipid Nano-Platforms as Non-invasive Paradigms for the Treatment of Ocular Inflammations: A Review

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ABSTRACT

Ocular inflammation is one of the common ophthalmic disorders triggered by different underlying causes and affecting both anterior and posterior segments of the eye. The functional barriers within the eye greatly impede the efficient ocular drug delivery, and hence the efficient relief of inflammation. The conventional treatment approaches are often limited to the topical applications of eye solutions, suspensions, or ointments, in addition to the intravitreal injections in case of diffuse inflammatory conditions. These strategies suffer from poor patient acceptance and compliance because of their ineffectiveness and lack of safety. During the past few decades, research attention was focused on the development of innovative ophthalmic dosage forms. Lipid nano-platforms had particular importance in drug delivery in terms of safety, biocompatibility, sustainment of drug release, enhancement of drug bioavailability, and hence patient compliance. This review attempts to highlight the current state of lipid-based nanocarriers as a promising non-invasive approach in the management of superficial and diffuse ocular inflammations with special emphasis on their reported outcomes, and also to focus on future perspectives to fill the gaps in this area.

Keywords: *eye; inflammation; lipid-based nanocarriers; topical delivery; non-invasive; anterior segment; posterior segment*

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Citation | Shakshak DH, Ishak RAH, Kamel AO, Mortada ND, 2021. Appraising the Competency of Lipid Nano-Platforms as Non-invasive Paradigms for the Treatment of Ocular Inflammations: A review. Arch Pharm Sci ASU 5(1): 71-96

DOI: 10.21608/aps.2021.73530.1056

Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.

Received 22 April 2021. **Accepted** 31 May 2021.

Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

Ocular drug delivery is one of the utmost interesting and motivating administration ways that confront pharmaceutical scientists. The eye anatomical and physiological structure hampers the entrance of active molecules to the anticipated site of action **(Fig. 1)**. The systemic and topical routes are engaged for ophthalmic drug delivery, though both have significant restrictions. Intravitreal drug administration is an invasive route often used to reach the site of action; however, it may be associated with numerous disorders such as endophthalmitis, vitreous hemorrhage, and retinal detachment particularly when applied recurrently. Therefore, this technique should not be employed except for special therapeutic indications **[1]**.

For the systemic route, penetration of the drugs into the tissues of the eye is extremely limited because, like the brain, the eye is protected by highly restrictive blood-ocular barriers (**Fig. 2**). These barriers have two parts:

the blood-aqueous barrier (BAB) and the blood-retinal barrier (BRB). The BAB, located in the anterior eye segment, is composed of endothelial cells in the uvea restricting the passage of water-soluble drugs from the blood circulation to the aqueous humor, while the BRB is positioned in the posterior chamber and composed of retinal pigment epithelium and the retinal capillaries, hindering the entrance of drug present in the blood to the retina **[2]**. This necessitates high drug concentration in the plasma to be able to reach its site of action increasing the incidence of side effects.

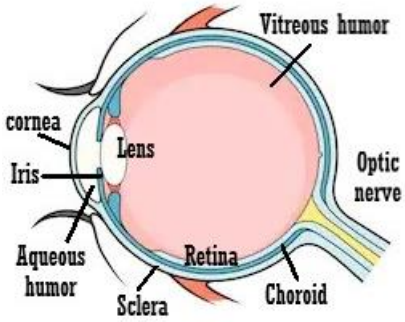

Fig. 1. Vertical eye section

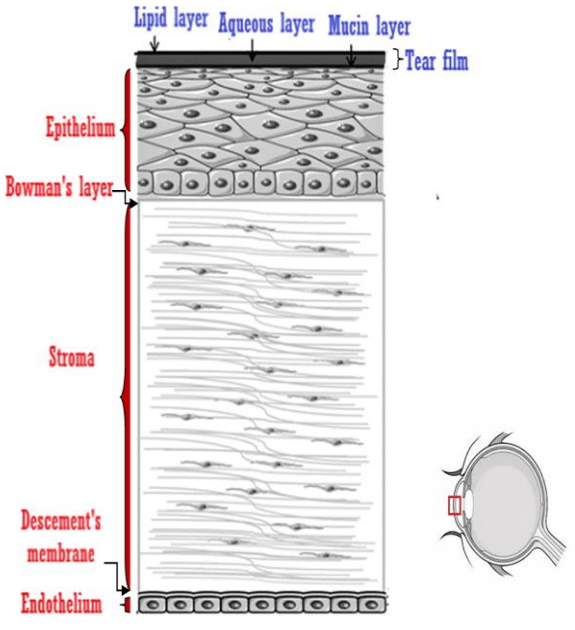

Fig. 2. Eye segments and barriers

For the topical route, using conventional eye drops results in only 5% of the administered dose reaching the eye. This is due to mainly two factors which are pre-corneal loss and limited corneal permeability **[3]**. Pre-corneal loss includes the elimination of the drug by lachrymal fluid and systemic absorption through the conjunctiva. It is known that the conjunctiva compared to the cornea is characterized by a larger surface area and higher permeability due to its leakier epithelium so the drug favors this pathway instead of the cornea **[4]**. The second factor is the highly impermeable cornea. The cornea comprises three leading membranes: epithelium, stroma, and endothelium as shown in **Fig. 3.**

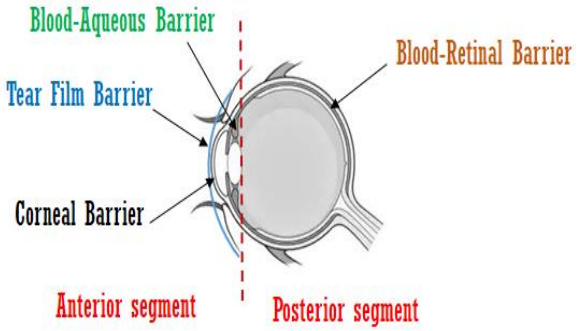

Fig. 3. Histological structures of the tear film and the cornea

The corneal epithelium is a lipophilic barrier that impedes the diffusion of hydrophilic drugs. It also acts as a discriminating barrier for small molecules and inhibits the passage of macromolecules through the paracellular route due to the presence of tight junctions between cells **[5]**. Under the epithelium, a highly hydrophilic layer called ‘the stroma’ is situated occupying about 90% of the corneal tissue. This layer presents an obstacle to the partitioning and entry of lipophilic drugs. The endothelium represents the deepest layer composed of flattened cells resembling those of corneal epithelium. This layer only conserves the hydration of the cornea without being a barrier

for drugs **[6]**. As being explained, the cornea carries both hydrophilic and lipophilic layers which act as barriers for both lipophilic and hydrophilic drugs, respectively.

In conclusion, the eye is a very challenging environment for drug delivery. Many barriers and mechanisms limit the drug penetration into the eye, including the lacrimal film, the blinking reflex, the nasolacrimal drainage system, the conjunctiva, the cornea, the sclera, the BAB, and the BRB which limit the bioavailability of drugs in the ocular tissues **[7]**.

2. Eye Inflammation

Inflammation is a common ocular disorder; however, it could be either superficial or diffuse. ‘Keratitis’ and ‘Conjunctivitis’ are the most familiar eye inflammatory diseases, in which the cornea and the conjunctiva are inflamed, respectively. The cornea is the transparent layer covering the pupil and the iris, while the conjunctiva covers the whites of the eye. Different types of keratitis are present; the superficial, the interstitial, and the posterior in which the corneal epithelium, stroma, and endothelium are affected, respectively **[8]**.

However, the most diffuse ocular diseases are 'uveitis and 'endophthalmitis'. In the former, the internal structures of the eye uvea. i.e. the choroid, the iris, and the ciliary body are inflamed, while the latter affects the intraocular fluid or tissue. Uveitis is classified into different types; the anterior uveitis, the most common type, also known as 'Iritis', affecting the front of the eye between the cornea and the iris, the intermediate uveitis, the posterior uveitis affecting the deep eye layers of the retina or the choroid, and the panuveitis in which the inflammation covers all uvea tissues.

All of these conditions cause eye redness, pain, blurred vision, floater appearance, and even loss of vision if not treated appropriately. This often occurs due to a bacterial, viral, or fungal infection, eye injury or surgery, or as a result of an autoimmune disease or an inflammatory disorder **[9]**.

The treatment approaches for ocular inflammatory diseases are usually based on the correction of the underlying causes. That's to say the topical applications of suitable antibiotics, antiviral or antifungals are mandatory, in addition to topical anti-inflammatory agents for instance corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs). Such topical administration is frequently not sufficient to improve the condition particularly for the diffuse conditions, thus the use of intraocular/intravitreal injections and/or oral anti-inflammatories is often applicable. However, these interventions suffer from unwanted side effects, high susceptibility to infection, and poor patient compliance **[10]**.

3. Conventional Ophthalmic Drug Delivery Systems

Till now the most common, acceptable, and comfortable route for ocular drug delivery to the patient is the topical one regardless of its limitations explained above. Improving drug retention, corneal permeation, and ocular bioavailability are the main goals of an ultimate ophthalmic formulation **[11]**.

3.1. Eye Drops

Although the diversity of ocular dosage forms, conventional eye drops are still the most popular. However, either solutions or suspensions suffer from being lost quickly due to the normal blinking action and the restricted volume of the pre-corneal area reaching about 7 µL **[12]**. The passive diffusion of the drug through the cornea depends on its concentration in the pre-corneal area. Moreover, a special category of patients particularly pediatrics and geriatrics reveal difficulty in the instillation of eye drops which led to the imprecision of the administered dose.

Therefore, inter-and intra-subject discrepancy in the therapeutic outcome is considered an unavoidable result.

Aiming to enhance the drug contact time, retention, and bioavailability, viscosity inducing agents and polymers such as hydroxypropyl methylcellulose, methylcellulose, and polyvinyl alcohol are commonly included in ocular solutions and suspensions to increase the viscosity of the preparation **[13]**, **[14]**.

Some mucoadhesive polymers, for example, gellan gum, xanthan gum, chitosan, hyaluronic acid, alginate, and cellulose derivatives, showed not only good potential to sustain drug release and increase its bioavailability, but also protective and healing properties to epithelial cells **[15]**, **[16]**. However, their effects on drug absorption are not dramatic as formulations are still liquid and can be eliminated by factors mentioned earlier for conventional eye drops **[17]**.

Stimuli-responsive systems have been formulated for ocular drug delivery. They are triggered by specific pH, ions, and temperature to be converted from a liquid phase to a gel. In contrast to viscous formulations, these *in situ* formed gels are easily dropped but stiffen when in contact with the tear film. Certain natural and synthetic polymers acquire such characteristics, for instance, gellan gum, cellulose acetate phthalate, and poloxamers **[18]**.

3.2. Eye Ointments

Eye ointments are prepared using a non-irritating blend of semi-solid and solid fatty bases which have a softening point near to the body temperature. Ocular ointments can enhance the drug's bioavailability and extend its release. However, eye ointments may cause blurred vision which in turn reduces patient compliance **[19]**, **[20]**. For this reason, they are often used as a means of nighttime medication.

To overcome the disadvantages of the conventional topical ocular delivery formulations several novel systems have been developed through extending the drug residence time in the cornea and conjunctival sac and improving corneal drug penetration to increase bioavailability and avoid systemic side effects.

In the same context, the compulsion to tailor advanced delivery systems is found relevant with the aim to enhance the ocular delivery and hence the effectiveness of anti-inflammatory drugs. This would greatly minimize the administration frequency of intraocular injections and oral antiphlogistic agents, resulting in reducing the risk and discomfort associated with injections and the side effects of the oral anti-inflammatories.

4. Innovative Platforms for Enhanced Ocular Drug Delivery

4.1. Ocular Inserts

Some solid polymeric discs have been designed in the form of eye inserts for ophthalmic drug delivery. These discs are inserted in the cul-de-sac showing prolonged retention times. This in turn reduces the frequency of administration and improves patient compliance in comparison to conventional eye drops. In addition, they permit accurate dosing and reduce systemic absorption and side effects **[21]**. However, patients often struggle to place a solid body in the pre-corneal area which led to system refutation.

4.2. Microparticles

Microparticles are either microspheres or microcapsules usually dispersed in an aqueous medium. They have an average particle size greater than 1 μm. when topically applied, these particles are maintained in the cul-de-sac allowing the drug release *via* diffusion and/or erosion mechanisms **[22]**. Being larger than nanoparticles they allow sustained drug release

but maybe less endured by the patients.

4.3. Nanoparticles (NPs)

Nanotechnology is the production of well-designed systems at the molecular or sub-micron scale. NP-based drug delivery systems are characterized by small particle size, narrow size distribution, and biocompatibility with low irritation potential. Hence, they may substantiate to be the preeminent therapeutics delivery platforms for the treatment of ocular diseases, and particularly eye inflammation. NPS refers to particulate drug delivery systems with particle sizes ranging between 1 to 1000 nm. They usually consist of biodegradable materials such as polymers, lipids, and even metals.

4.3.1. Polymeric NPs

They are made of natural polymers such as chitosan, gelatin, sodium alginate, and albumin or synthetic polymers which may be biodegradable that undergo hydrolysis in tears such as polylactic acid (PLA), poly alkyl cyanoacrylates (PACA), poly-ε-caprolactone (PCL), and polylactic-co-glycolic acid (PLGA). The drug may be dispersed in the polymer matrix forming nanospheres or surrounded by the polymer membrane forming nanocapsules. However polymeric NPs suffer from many disadvantages as the use of organic solvents in their preparation methods, polymer cytotoxicity, and the difficulty of scaling up the production process **[23][24]**.

4.3.2. Lipid Nanocarriers

These lipidic carriers have been widely employed for the delivery of a variety of active moieties by various routes of administration. They demonstrate many benefits in comparison to polymeric NPs. They mimic the lipid components of the physiological environment, hence have good biocompatibility **[23]**. In particular, lipid nanocarriers are a versatile delivery tool, comprising vesicular lipid systems and lipid-based NPs. The different types of lipid nanocarriers are shown in **Fig. 4** and discussed in the following sections. During the last decade, many formulation attempts *via* lipid-based nanocarriers were accomplished for alleviating ocular inflammation by the delivery of therapeutics to the anterior eye segment; these are collected and summarized in **Table 1**.

Table 1. A collective table summarizing the published works on the ocular delivery of lipid-based nano-sized platforms for the treatment of inflammatory diseases affecting the anterior eye segment

Lipid Platform type(s)	Therapeutic Agent and its Concent.	Ocular inflammatory disease	Composition(s) of the optimized formula(e)	Characteristics of the optimized formula(e)	Main findings/results	Ref.
NE-in-Ion sensitive *In situ* Gel	Terbinafine Hydrochloride (0.5% w/v)	**Fungal Keratitis**	NE: - Miglyol® 812 as oil (5% w/w) - Cremophor EL: Polyethylene glycol 400 (1:2) as Surfactant:Co-surfactant blend (55% w/w) - Water (40% w/w) In situ Gel: Gellan Gum (0.2% w/v)	PS < 30 nm	• Zero-order release kinetics • Least ocular irritation potential • High Cmax and retarded Tmax • Extended residence time • Increased drug BAV in aqueous humor compared to oil solution	**[70]**

Cationic NE	Ibuprofen	**Inflammation due to eye dryness**	- Ibuprofen (0.2% w/w) - Miglyol® 812 (2.5%w/w) - Lecithin (0.05% w/w) - Kolliphor® EL (0.25% w/w) - Glycerol (2.5% w/w) - Water (94.45% w/w) - Chitosan as cationic mucoadhesive polymer (0.05% w/w)	PS = 175.1 nm PDI = 0.127 ZP = +24.6 mV EE = 98.73% pH = 4.41 Viscosity = 1.9 mPa.s Osmolarity = 300.3 mOsm/kg Surface tension = 35.7 mN/m	● The developed cationic NE increased drug retention, and improved stability in the tear film ● The cationic NE showed mucoadhesive properties when mixed with mucin dispersion. ● Biocompatibility of the cationic NE was confirmed using 3D HCE-T cell-based model and *ex vivo* porcine cornea.	**[71]**
Modified o/w ME	Ofloxacin (0.3% w/w)	**Bacterial Keratitis**	ME: - Oleic acid as oil (15%) - Tween® 80: Ethanol as surfactant: Co-surfactant mixture (2:1) (60%) - 0.5 N NaOH aqueous solution (25%) Modification: 0.75% chitosan oligosaccharide lactate (COL)	PS = 146.8 nm PDI = 0.193 ZP = -0.561 mV pH = 7 Viscosity = 90.01 cP	Compared to non-modified ME, o/w ME with COL showed: ● slower release rate ● Lower permeation rate ● Higher anti-bacterial activity ● Similar pre-ocular residence time	**[72]**
w/o ME	Moxifloxacin (0.5% w/w)	**Bacterial keratitis**	- Isopropyl myristate as oil (33% w/w) - Tween 80/Span 2 as surfactants (22.8/34.2% w/w) - Acetate buffer (10% w/w)	PS = 36.25 nm PDI = 0.25 Drug content = 98.2% pH = 6.46 RI = 1.449	● Longer pre-corneal residence time ● Sustain drug release ● Enhanced efficiency in treatment of bacterial keratitis in animal models compared to marketed drug solution	**[73]**
Liposomes	Voriconazole (VOR)	**Fungal Keratitis**	Soy phosphatidylcholine (PC) at VOR:PC molar ratio 7.2:40 mM [without 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) nor cholesterol]	PS = 116.6 nm PDI = 0.17 ZP = -7.35 mV EE = 86.8%	● Optimized formulation proved safety and non-irritancy using HET-CAM's test. ● Liposomes permitted VOR permeation of about 47.85 $\mu g/cm^2$ after 30 min across porcine cornea, considered higher than MIC against many fungi species isolated from clinical patients.	**[67]**
Liposomes-in-biodegradable hydrogel	Dexamethasone (DEX) Moxifloxacin (MOX)	**Infectious Keratitis**	Liposomes: - MOX (50 mg), Cholesterol (7.7 mg) and DSPC (110.6 mg) at molar ratio = 14:15:2 - DEX (5 mg) Biodegradable hydrogel: - Gelatin (3%) - Alginate (1%) - Collagen (0.1%) cross-linked with a 3% $CaCl_2$ solution	Liposomes: PS = 213.4 nm PDI = 0.266 ZP = -4.8 mV EE = 94.5% Drug Content = 18.1%	● Hydrogel containing MOX/DEX liposomes showed a sustained release for at least 12 h. ● The composite system demonstrated safety on corneal epithelial cells. ● The composite system inhibited bacterial growth and improve corneal wound healing *in vivo*.	**[65]**

Niosomes	Lemofloxacin HCl	**Bacterial Conjunctivitis**	Tween® 60 + cholesterol at 1:1 molar ratio	PS = 176 nm nm ZP = -40.7 mV EE = 68.41%	• Optimized niosomes showed no signs of ocular toxicity. • Microbiological susceptibility test demonstrated significantly higher % inhibition of *S. aureus* and $AUC_{0\text{-}12\,h}$ of optimized formula compared to the commercial product. • The infected eyes revealed complete eradication of *S. aureus* and healing.	**[74]**
Niosomal gel - **w/o ME** -	Fluconazole (0.3% w/w)	**Fungal Keratitis**	<u>Niosomal Gel:</u> Span® 60 + cholesterol at 5:5 molar ratio in 1% w/w carbopol® 934 <u>w/o ME:</u> - Isopropyl myristate as oil (45%) - Surfactant:Co-surfactant mixture (40%) - Tween® 80: PEG 400 (3:1) - Distilled water (15%)	<u>Niosomal Gel:</u> PS = 117.13 nm ZP = -45.37 mV EE = 63.21% pH = 6.35 Viscosity = 1419 mPa.s Drug Content = 99.42 (%w/w) <u>w/o ME:</u> PS = 59.93 nm ZP = -31.9 mV pH = 5.63 Viscosity = 312 mPa.s Drug Content = 101.3 (%w/w)	• More sustain drug release from niosomal gels than niosomes and ME • Niosomal gel revealed higher ocular BAV than ME by ≈2-fold	**[75]**
Dual purpose Niosomes-in-gel	Natamycin (NAT) Ketorolac tromethamine (KT)	**Fungal Keratitis**	<u>NAT-niosomes:</u> - NAT (0.2%) - Span 60:Cholesterol (1:0.5) molar ratio <u>NAT-niosomes/0.5%KT gel:</u> 2% HPMC E4 (F5) 4% Na CMC (F8)	<u>NAT-Niosomes:</u> PS = 181.75 ± 0.64 nm ZP = -58.95 ± 0.64 mV EE = 96.43 ± 0.24% Drug Loading = 14.75 ± 0.009% Release = 77.49% over 24h	• NAT-loaded niosomes/0.5%KT gel formulae revealed retardation *in vitro* release, compared to marketed-product (NATACYN®) and NAT-loaded niosomes up to 57.32%. • *In vivo* studies showed the superiority for F8 in corneal infiltration and hypopyon level in comparison with F5 and combined marketed products (NATACYN® and Ketoroline®).	**[76]**
Proniosomal gel	Lomefloxacin HCl (LXN)	**Bacterial Conjunctivitis**	- LXN (10 mg) - Surfactant mixture (500 mg) = Span® 60:Tween® 60 9:1(w/w) - Cholesterol (50 mg) - Ethanol (0.16 ml) - Water (0.05 ml)	PS = 187 nm PDI = 0.11 ZP = -58.03 mV EE = 83.95%	• Selected LXN-proniosomal gel revealed safe by the irritancy test. • Drug-loaded proniosomal gel demonstrated enhanced antibacterial activity using the susceptibility test and improved efficacy of induced conjunctivitis compared to the marketed LXN eye drops.	**[77]**

SLN	Calendula officinalis extract	**Epithelium repairing**	- Stearic acid as solid lipid (0.07 mmol) - Epikuron 200, consisting mainly of phosphatidylcholine, as emulsifier (0.014 mmol) - Sodium taurodeoxycholate as cosurfactant (0.066 mmol) - ultrapure water as the continuous phase (11.11 mmol)	PS = 67 nm PDI = 0.2 ZP = - 48 mV EE = 70%	*In vitro* cytotoxicity and wound healing effect of Calendula-loaded SLN showed efficiency in comparison to the free extract on conjunctival epithelial cells.	**[78]**
SLN	Levofloxacin	**Bacterial Conjunctivitis**	- Stearic acid as solid lipid (6.31%) - Tween® 80 as surfactant (3%) - Sodium deoxycholate as cosurfactant (1%) - ultrapure water as the continuous phase	PS = 237.82 nm EE = 78.71% pH = 6.4	● Optimized SLN showed trans-corneal drug permeation with a flux of 0.2493 μg/cm^2/h. ● Optimized drug-SLN revealed antibacterial activity against *S. aureus* and *E. coli* comparable to marketed eye drops. ● Optimized drug-SLN was proved to be safe and non-irritant using HET-CAM test.	**[66]**
Modified NLC	Ofloxacin (OFX) (0.3% w/w)	**Bacterial keratitis**	NLC: - Compritol® HD5 ATO as solid lipid (0.09 g) - Oleic acid as oil (0.18 g) - Tween® 80 as surfactant (3.88 g) - Ethanol as co-surfactant (1.94 g) - Ultrapure water (3.91 g) Modification: 0.75% chitosan oligosaccharide lactate (COL)	Modified NLC prepared with: ❖ warm o/w ME method: PS = 10.8 nm PDI = 0.468 ZP = +5.84 mV EE = 99.925% Viscosity = 52 cP ❖ high shear homogenization method: PS = 238.3 nm PDI = 0.301 ZP = +36.1 mV EE = 99.971% Viscosity = 43.6 cP	Both modified NLC showed: ● Improved pre-ocular residence time ● Sustained drug release ● Boosted drug BAV	**[79]**
NLC-based insert			NLC: - Compritol® HD5 ATO as solid lipid (0.73%) - Oleic acid as oil (1.47%) - Tween® 80 as stabilizer (0.73%) - Ultrapure water (97.07%) Insert: - Glycerin as plasticizer (5%) - 0.75% chitosan oligosaccharide lactate (COL)	NLC: PS = 153.5 nm PDI = 0.188 ZP = - 4.63 mV EE = 98.44%	● Selected OFX insert was more bioadhesive *in vitro*. ● The retention time lasted for 24 h ● Cmax was increased six-fold compared to commercial eye drops. ● Rabbits infected with S. aureus and developed keratitis was effectively treated with the selected OFX insert ● Recovery was attained afrter 7 days without any signs of conjunctival redness and corneal opacity	**[80]**

Coated NLC	Acyclovir (ACV)	**Herpes Keratitis**	NLC: - Solid lipid: Compritol® 888 ATO (58% w/w) - Liquid lipid: Lauroglycol® 90 - Surfactant: Tween® 40 Modification: 0.5% chitosan	PS = 457.30 ± 44.38 nm ZP = + 28.10 ± 0.72 mV EE = 84.76 ± 1.23%	• Increased *in vitro* antiviral activity by 3.5-fold by cellular internalization • Improved corneal BAV by 4.5-fold compared to marketed ACV ointment.	**[81]**
Modified NLC	Amphotericin B (AmB)	**Fungal Keratitis**	NLC: - Compritol® 888 ATO (60 mg) - Lecithin (50 mg) - Soybean oil (50 mg) Modification: Chitosan oligosaccharides (CH)	PS = 185.4 nm PDI = 0.2 ZP = + 27.1 mV EE = 90.9%	• Sustained drug release was achieved *in vitro* • AmB-CH-NLC showed enhanced ocular pharmacokinetics and BAV • AmB-CH-NLC revealed improved corneal penetration with no signs of irritation in rabbits' eyes	**[82]**
PEGylated NLC	Natamycin (NAT-0.3%)	**Fungal Keratitis**	- NAT (0.3% w/v) - Castor oil (1% w/v) - Precirol® ATO 5 (1.5% w/v) - Glycerin (2.25% w/v) - Span® 80 (0.11% w/v) - Poloxamer® 188 (0.25% w/v) - Tween® 80 (0.75% w/v) - sodium salt of mPEG-2K-DSPE (1.5% w/v)	PS = 241.96 nm PDI = 0.406 EE = 95.35% DL = 6.45% Drug content = 97.85%	• NAT-PEG-NLCs showed higher *in vitro* transcorneal drug permeability and flux than that of the marketed suspension (Natacyn®). • NAT-PEG-NLC (0.3%) revealed enhanced NAT delivery across the intact cornea similar to the commercial product (5%) *in vivo*.	**[68]**
NLC in thermoresponsive hydrogel	Sertaconazole nitrate (STZ) (16 mg)	**Fungal Keratitis**	NLC: - Stearic acid (SA) (80 mg) - Labrafac®:SA ratio = 10 - W/O ratio = 10 - Pluronic® F127 as surfactant (0.5%) *In situ* hydrogel: Pluronic F127/HPMC K4M (19%:0.5%)	NLC: PS = 272.4 nm PDI = 0.31 ZP = + 12.9 mV EE = 89.97%	• STZ-NLC revealed greater antifungal effect and corneal permeation than free drug and *in situ* gel. • Corneal STZ permeation from NLC-containing *in situ* gel was comparable to that of free drug	**[83]**
Cubosomes	Sertaconazole nitrate (STZ) (5 mg/ml)	**Fungal Keratitis**	- DL-α-monoolein as lipid (15% w/w) - Brij® 58 as stabilizer (0.85% w/w) - Homogenization time: 10 min	PS = 216.55 nm PDI = 0.229 ZP = + 34 mV EE = 94.5%	STZ-cubosmes showed: • Good mucoadhesive behavior • Enhanced corneal permeation *ex vivo* and *in vivo* compared to drug suspension • Safety on the corneal tissues *in vivo*	**[69]**

ACV, Acyclovir; AmB, Amphotericin B; AUC, Area under the curve; BAV, Bioavailability; $CaCl_2$, Calcium Chloride; CH, Chitosan oligosaccharide; COL, Chitosan oligosaccharide lactate; Cmax, Maximum drug concentration; DEX, Dexamethasone; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; EE, Entrapment efficiency; HET-CAM: hen's egg-chorioallantoic membrane; KT, Ketorolac tromethamine; LXN, Lomefloxacin HCl; ME, Microemulsion; MIC, Minimum inhibitory concentration; mPEG-2K-DSPE,1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine with conjugated methoxyl poly(ethylene glycol) 2000; MOX, Moxifloxacin; NaOH, Sodium hydroxide; NAT, Natamycin; NE, Nanoemulsion; NPs, Nanoparticles; NLC, Nanostructured lipid carrier; OFX, Ofloxacin; PC, Soy phosphatidylcholine; PDI, Polydispersity index; PEG, Polyethylene glycol; PS, Particle size; SA, Stearic acid; SLN, Solid lipid nanoparticles; STZ, Sertaconazole nitrate; Tmax, Time at which maximum drug concentration occur; VOR, Voriconazole; ZP, Zeta potential; 3D HCE-T model, **3D** human corneal epithelial tissue model

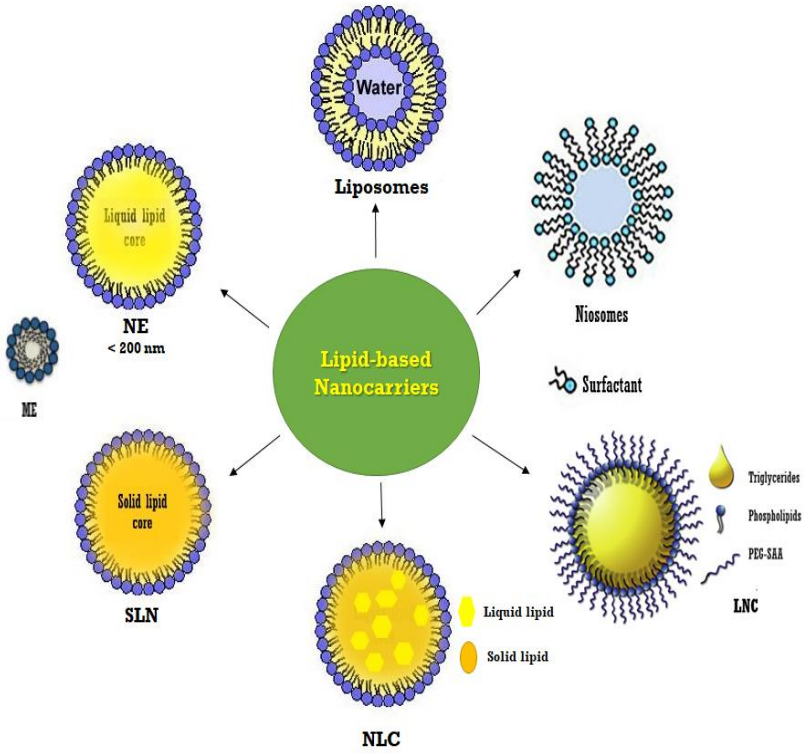

Fig. 4. Schematic diagram illustrating the various types of lipid-based nano carriers. NE: Nanoemulsion; ME: Microemulsion; SLN: Solid Lipid Nanoparticles; NLC: Nanostructured Lipid Carriers; LNC: Lipid Nanocapsules

5. Types of Lipid-Based Nanocarriers

5.1. Vesicular Colloidal Systems

Vesicular delivery systems include liposomes which are phospholipid-based vesicles and niosomes which are non-ionic surfactant-based vesicles. Ethoniosomes, transferases, emulsions, and cubosomes were also developed as promising novel vesicular delivery systems.

Liposomes are microscopic and submicroscopic vesicles with sizes ranging from l0 nm to 20 µm. The vesicles consist of one or more concentric spheres of membrane-like lipid bilayers separated by water or aqueous buffer compartments **[25]**. They can be classified into multilamellar vesicles, small unilamellar vesicles, large unilamellar vesicles, and multivesicular liposomes**.** However, liposomes suffer from poor chemical and physical stability leading to liposome aggregation and drug degradation during storage **[26]**. This is due to the hydrolysis of the ester bindings of the phospholipids and the oxidation of the unsaturated fatty acids.

The liposomes were extensively exploited during the last 30 years for their potential application in ocular drug delivery. The effect of surface charge and size of vesicles on the trans-corneal flux of prednisolone acetate was investigated *in vitro* and *in vivo*. The positively charged multilamellar liposomes (p-MLV) revealed a reduced prednisolone flux across the cornea compared to neutral multilamellar vesicles (n-MLV), however, the small size of nanosized unilamellar vesicles (ULV) enhanced the drug permeation. The latter liposomal system also showed higher prednisolone concentration in aqueous humor than in the case of n-MLV **[27]**.

A cationic nano-liposomal system was designed for the sake to carry gold nanoparticles

capped with flucytosine for the treatment of intraocular inflammation caused by fungal infection **[28]**. The prepared nanocomposites showed deeper penetration ability through tracking nano-gold as a contrast agent by tomography imaging. Higher antifungal activity was also demonstrated in rabbit models, proving the effectiveness of the nanosized liposomal carrier in reaching the posterior eye segments and thus treating endophthalmitis caused by fungal infections.

A fluorescent-labeled liposomal formulation was proven to be uptaken by human corneal epithelial cells for 72 h after 1 h exposure and uniformly distributed across *ex vivo* porcine corneal tissues after 5 min contact. This blank liposome was considered effective in supplement tear lipids and hence indicated as artificial tears for dry eyes. The assimilation of an anti-inflammatory agent as Medroxyprogesterone acetate in the same liposomal platform was found to improve the ocular inflammatory condition by reducing cytokine production, demonstrating better results when compared to the non-liposomal drug formulation **[29]**.

Lately, liposomes were considered for loading antibacterial agents e.g., Ciprofloxacin (Cipro) for the treatment of endophthalmitis as a result of a bacterial infection or after eye surgery **[30]**. The results revealed the increase in Cipro ocular bioavailability and anti-bacterial effect after instillation of Cipro-liposomes and Cipro-coated liposomes with Carbomer compared to marketed Cipro eye drops. This was confirmed by the higher drug concentrations detected in aqueous and vitreous humor treated with medicated liposomes than Cipro minimum inhibitory concentration (MIC) against *S. aureus* and *P. aeruginosa*.

Liposomes were also used to carry the immunosuppressant Sirolimus for the treatment of posterior segment eye diseases (PSED) **[31]**. Sirolimus has an inhibitory activity on T-cell proliferation and the release of inflammatory cytokines. The conventional liposomes were modified by adding polyols to their bilayer structure. This adaptation improved the flexibility and deformability of the vesicles, enhanced the drug transport through the blood-retinal barrier, and thus proved the efficiency of the altered liposomes in the treatment of PSED.

Niosomes are analogous to liposomes and serve also as promising drug carriers. They are vesicles based on non-ionic surface-active agents that are self-assembled in water forming bilayer structures **[32]**, **[33],** therefore they are capable to load both hydrophilic and lipophilic actives **[32–34]**. However, transferases are combined vesicles that emerged from both liposomes and niosomes. They are phospholipid-based vesicles in which an edge activator or a single chain surfactant was added affording high flexibility and deformability.

A modified liposomal system, so-called ethoniosomes, was developed integrating both Span 60 and ethanol in the same construct, and used for loading prednisolone acetate (PA) and prednisone sodium phosphate (PSP). The novel vesicles showed good ocular tolerability without irritancy. The respective nanovesicles exhibited higher ocular bioavailability than PA suspension and PSP solution revealing a significant anti-inflammatory effect. These medicated lipid nanocarriers succeeded to prevent the elevation of the intraocular pressure, being the main side effect of prednisolone, when compared to the corresponding eye drops **[35]**.

The effect of different vesicular systems; liposomes, penetration enhancer-enrich vesicles, and transferases on the ocular drug pharmacokinetics was recently exploited by Ashraf *et al,* 2018 **[36]**. Baicalin was the natural flavonoid used due to its anti-inflammatory and antioxidant activity. Baicalin-loaded sodium

taurocholate-based transferases showed the fastest onset of action, expressed by a short Tmax, however, medicated liposomes exhibited the highest absorption extent in terms of Cmax and AUC in the aqueous humor. All prepared vesicles demonstrated an increase in drug bioavailability by 4-5-folds in comparison to baicalin solution. The study results confirmed the potential application of baicalin-loaded vesicles in the treatment of different ophthalmic maladies including inflammation. The authors also suggest further ophthalmic applications of the developed vesicles in the treatment of cataract and diabetic neuropathy.

Emulsomes are novel vesicle-derived systems composed of a solid fat core surrounded by a phospholipid layer. In contrast to liposomes, the hydrophobic drugs are localized not only in the bilayer structures but also within the inner hydrophobic matrix, offering a high drug payload. These novel nanocarriers were recently used for the non-invasive delivery of triamcinolone acetonide to the posterior ocular segment **[37]**. The biosafety of the developed vesicles was confirmed by the *in vitro* corneal cellular studies. Nile Red-loaded emulsions showed diffuse fluorescence distribution to the inner and outer plexiform retinal layers. The results proved the suitability of the novel system to treat posterior eye segment diseases by a simple topical instillation.

Cubosomes, are individual nanoparticles that result from the colloidal dispersion of bicontinuous cubic liquid crystalline structures in water using appropriate surfactants **[38]**. They are characterized by a unique and thermodynamically stable isotropic structure, consisting of a three-dimensional curved bicontinuous lipid bilayer with two congruent networks of water channels **[39]**. Cubosomes display low viscosity, high heat stability, and a large surface area. They can also encapsulate both hydrophilic and hydrophobic drugs and are ideal candidates for delivery of pharmaceuticals, due to their inexpensive raw materials, increased solubilization benefit of the active ingredient, and their potential for releasing their ingredients in a sustained manner **[40]**. It should be noted that the structure and composition of ribosomes are similar to that of biological membranes; this allows lipid carriers to more easily bind with lipid bilayers of the corneal epithelial cells. For all these physicochemical characteristics, they constitute excellent candidates for ocular drug delivery systems **[41–44]**.

According to a recent study, beclomethasone disproportionate (BDP) cubosomes were prepared using the top-down technique using glyceryl monooleate (GMO) as the lipid and two different stabilizers; poloxamer® 407 and solulan® C24. The prepared cubosomes were of nano-sizes (100 nm-278 nm), and EE% was around 94%. Transcorneal permeation parameters; P_{app} and flux and $AUC_{0\text{-}10h}$ markedly enhanced by up to 4-, 5.8-and 5.5-fold respectively, compared to the control BDP-suspension formulation. This study suggested that cubosomes/Cubo-gel could be an auspicious ocular delivery system for BDP to effectively treat uveitis **[45]**.

5.2. Lipid-Based NPs

Besides liposomes and other phospholipid or amphiphiles-based vesicles, several lipid-based nanocarriers have been tailored using solid and liquid lipids forming matrices during the last three decades.

Lipid-based NPs are characterized by rigid structures with better stability over vesicular systems. In addition, these lipidic NPs could enhance the loading capacity as well as the stability of the entrapped active ingredient(s) by preventing its escape during storage.

Similar to polymeric NPs, lipid-based nanocarriers can be divided into two main categories: nanospheres, in which the drug is distributed uniformly in a homogeneous or heterogeneous structured lipid matrix, and nanocapsules, which display a characteristic core-shell structure where the drug is localized mainly in the inner oily core and to a lesser extent in the hard lipid shell.

Lipid nanoemulsions are also considered as a prototype for NP formation. Similarly, lipid NPs can be prepared from microemulsions **[46]**.

5.2.1. Nanoemulsions (NEs) and Microemulsions (MEs)

The NEs and MEs are both nano-sized isotropic emulsions prepared from two immiscible liquids usually water and oil, and stabilized by an emulsifying agent. e.g. a surfactant/co-surfactant blend. The droplet sizes of both systems are typically < 200 nm, or even lower than 100 nm, particularly for MEs. As reported, the difference between them is that NEs are kinetically stable, while MEs are thermodynamically stable.

Ocular NEs and MEs are simple systems characterized by ease of preparation and scaling up using high-pressure homogenization, however, few were developed for the treatment of eye inflammation. The fact is attributed to the fear of irritation due to the high proportion of surfactant/co-surfactant used in these systems. Recently, Smail *et al.* **[47]** investigated the ocular irritation potential of different surfactants, co-surfactants and oils usually employed in the fabrication of NEs. Many components (11 surfactants, nine cosurfactants, and five oils) were investigated as potential excipients for the preparation of ketorolac tromethamine (KT) ocular NE by applying Hen's Egg Test-ChorioAllantoic Membrane (HET-CAM) assay. Among the excipients examined in this study, a group of non-ionic surfactants was selected (Tween 20, Tween 60, Tween 80, Span 20, Span 80, Cremophor RH 40, Cremophor EL, Labrasol ALF, Labrafil M 2125 CS, Labrafil M 1944 CS, and Labrafil M 2130 CS) with different HLB values. Cosurfactants included (ethylene glycol, propylene glycol, butylene glycol, pentylene glycol, hexylene glycol, dimethyl isosorbide, 2-butyl-2-ethyl-1,3-propanediol, (2,3-butanediol), and Transcutol P), while triacetin, ethyl oleate, Labrafac PG, Labrafac lipophile WL 1349, and isopropyl myristate were used as oils. All surfactants, except Labrasol ALF, revealed safety without any irritancy. The majority of cosurfactants were slightly irritant, butylene glycol was a moderate irritant, pentylene and hexylene glycols were strong irritants. Triacetin and Labrafac lipophile WL 1349 would be the oils of choice.

The NSAID Flurbiprofen axetil (FA) was included in a novel NE composed of castor oil droplets dispersed in an aqueous phase and stabilized by Tween 80. The solubilization of the prodrug FA in the dispersed phase conserved the drug stability. The prepared NE demonstrated improved drug concentrations in aqueous humor by about seven-fold compared to the FA oil solution. The mean ocular residence time of FA increased with the increase of oil concentration. The prepared FA NE showed better anti-inflammatory activity with ocular biocompatibility than the commercial Flurbiprofen eye drops [48]**.**

For the last two decades, MEs were used to deliver corticosteroids to the eyes for improved drug permeation and diffusability to the deep ocular tissues. The safety and tolerability of ocular ME were confirmed. In comparison to conventional eye drops, ME showed greater penetration of dexamethasone (DEX) to the anterior ocular segments with a prolonged drug release. This in turn recommended lowering the

frequency of daily applications [49]**.** A modified ME was also developed by coating with chitosan (Cs) **[50]**. This cationic ME revealed high mucoadhesive properties with an improved anti-inflammatory effect of DEX in a uveitis-induced rabbit eye model when compared with a marketed drug suspension.

Recently, the proficiency of triamcinolone acetonide (TA)-loaded ME as an ophthalmic delivery system was demonstrated for the treatment of uveitis. The optimized ME formulation was prepared using oleic acid as the oil, Cremophor EL, and propylene glycol as a surfactant and co-surfactant (1:1), and water (15:35:50% *w/w*, respectively). This formula was found stable and showed acceptable pH, viscosity, conductivity, droplet size (211±1.4 nm), and zeta potential (-25±1.7 mV), and almost complete *in vitro* drug release within 24 h. The developed TA-loaded ME showed superior therapeutic effectiveness in the experimentally uveitis-induced rabbit model compared to commercial suspension (Kenacort®-A) when applied topically or by subconjunctival injection. This was confirmed by clinical examination, white blood cell count, protein content, and histopathological examination **[51]**.

5.2.2. Solid Lipid Nanoparticles (SLN)

The SLN is solid lipids-based nanospheres with a particle size up to 1 micrometer (μm). They are generally formed of solid lipid(s) dispersed in an aqueous medium and stabilized by one or more surfactant types from various classes. The solid lipids may include triglycerides (e.g., tripalmitin), mono-/di-glycerides, fatty acids (e.g., palmitic acid), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate).

SLN is considered physicochemically stable carriers which can be easily subjected to scaling up and thus manufactured on a large industrial scale, taking into account the low cost of their components. They can be prepared by various techniques such as high-pressure homogenization (HPH), high shear homogenization, ultrasonication, and solvent injection **[52]**, **[53]**.

The SLN was a good candidate to efficiently deliver celecoxib with prolonged residence time over corneal surfaces **[54]** and triamcinolone acetonide (TA) to anterior and posterior ocular tissues **[55]**. The medicated SLN can be further incorporated into *in-situ* gel to improve drug transport to various ocular tissues. This was revealed by the composite TA-SLN/gellan gum platform which displayed higher drug concentrations in tear fluid, aqueous and vitreous humor, corneal tissues than the drug suspension **[55]**.

5.2.3. Nanostructured Lipid Carriers (NLC)

The NLC is the second generation of lipid NPs that consist of a blend of solid and liquid lipids. The inclusion of liquid lipid (oil) into the solid lipid matrix causes depression in the melting point of the latter component; however, the produced particles maintain their solidified structure at body temperature. NLC showed an increased drug loading without being expulsed during storage [56].

Various NLC types are formed based on the preparation technique and the type of lipid mixture, for instance imperfect, amorphous, and multiple-type. The former type is generated by the addition of small quantities of liquid lipids preventing the crystallization of solid lipids. Also, the amorphous type is produced by blending specific lipids, for instance, hydroxyoctacosanyl hydroxy stearate and isopropyl myristate as the solid and liquid lipids, respectively. However, blending the solid lipid with large oil content causes the development of multiple types where small compartments inside the solid matrix are formed.

NLC can be effectively produced by HPH and solvent injection techniques, forming lipid dispersions with high solid content ranging from 30 to 80% **[53]**, **[57]**.

The medicated NLC is considered a potential non-invasive alternative treatment of endophthalmitis instead of intraocular injections of antibiotics as declared by **[58]**. Moxifloxacin-NLC loaded in *in situ* gel succeed to enhance *ex-vivo* trans-ocular drug permeation and retention two-fold when compared to free drug-*in situ* gel.

Although corticosteroids are commonly indicated for the treatment of inflammation and the alleviation of fibrosis generated after corneal burns due to chemicals, Rapamycin (Rapa), an immunosuppressant drug, was studied for this novel application *via* its inclusion in NLC. Rapa loading into NLC resolved the drug limitations such as hydrophobicity and instability in the presence of heat, light, and extreme pH conditions. The uptake of the developed Rapa-NLC was enhanced into fibroblasts, proving significant antifibrotic and anti-angiogenetic corneal activity when injured, and hence conserving corneal transparency **[59]**.

Being a biocompatible carrier, a cationic NLC (cNLC) system was recently employed for the delivery of triamcinolone acetonide (TA) to deeper ocular tissues to replace the intravitreal TA injections associated with the risk of ocular hemorrhage and infections. cNLC by stearyl amine was supposed to increase drug residence time and uptake *via* the electrostatic attractions between the positively charged nanocarriers and the mucus layers of opposite charges. The corticosteroid-loaded cNLC showed an enhanced trans-corneal permeation by 2-fold and higher anti-inflammatory activity than TA suspension. The authors declare the suitability of the developed platform as a safe promising option for the treatment of uveitis at lower TA concentration (0.1%) **[60]**.

5.2.4. Lipid Nanocapsules (LNC)

The LNC is the last generation of core-shell type lipid nanocarriers comprising an oil core and an external shell, composed of solid lipids and emulsifiers. In contrast to NLC of multiple types which consist of a solid lipid matrix with several oil compartments, LNC holds a single oily core in which the drug dissolves, surrounded by a thin solid coat **[61]**.

Recently, the corticosteroid triamcinolone acetonide (TA) was loaded into LNC for the target to cure eye disorders including inflammation and angiogenic processes. The nanocapsules were prepared by the phase inversion temperature (PIT) technique where successive three cycles of heating and cooling were applied. The outer nanocapsule shell was composed of phospholipids (Lipoid® S75-3) and Kolliphor® HS, while the inner oily core comprising a mixture of Labrafac® WL1349 and either oleic acid (OA) or Captex® 500p as co-surfactant to improve the solubility of TA. The use of co-surfactants showed a significant increase in drug loading capacity > 200 µg/g. The optimized TA-loaded LNC were tested for safety on human corneal epithelial cells and showed a high anti-inflammatory effect. This was confirmed by the reduction in interleukin-6 levels and the improvement of inflammatory signs in an endotoxin-induced uveitis rabbit model, hence suggesting its prospective applicability for the treatment of various eye diseases **[62]**.

Lately, a novel hybrid formulation based on lipid nanocapsules containing bevacizumab (BVZ), an effective therapeutic antibody, on the surface and triamcinolone acetonide (TA) in the inner core (BVZ-TA-LNC) intended to improve ocular therapy. BVZ-TA-LNC were nanometric in size (102 nm) with a negative surface potential (-19 mV) and encapsulating 56% of TA in the lipid core. BVZ-TA-LNC tended to prevent the endothelial cell migration and the capillary

formation induced by the vascular endothelium growth factor (VEGF). This novel hybrid system may be a promising candidate for the treatment of eye disorders associated with inflammation and/or neovascularization **[63]**.

5.2.5. Lipid-Polymer Nano-composite Platforms

The amalgamation of lipid and polymer in the same nanoconstruct was seldom investigated for ocular drug delivery, particularly for the treatment of inflammatory disorders. However, Du Toit *et al.* **[64]** proved the efficiency of composite lecithin-Chitosan/poly (ε-caprolactone) nanosystem compared to pure polymeric NPs and drug suspension as well in terms of permeation, retention, cellular uptake, and anti-inflammatory activity. The authors reported the enhanced indomethacin delivery and efficacy to the posterior eye segments in favor of composite NPs.

6. Experimental Evaluation of Ocular Lipid-Based Nanocarriers

Many ocular experiments are often performed aiming to assess the effectiveness of the tested lipid nano-formulations applied for the treatment and management of eye inflammation. These protocols vary from *in vitro* bacterial or cellular culture, *ex vivo* corneal experiment, and *in vivo* animal models as explained in the following section and illustrated in **Fig. 5**.

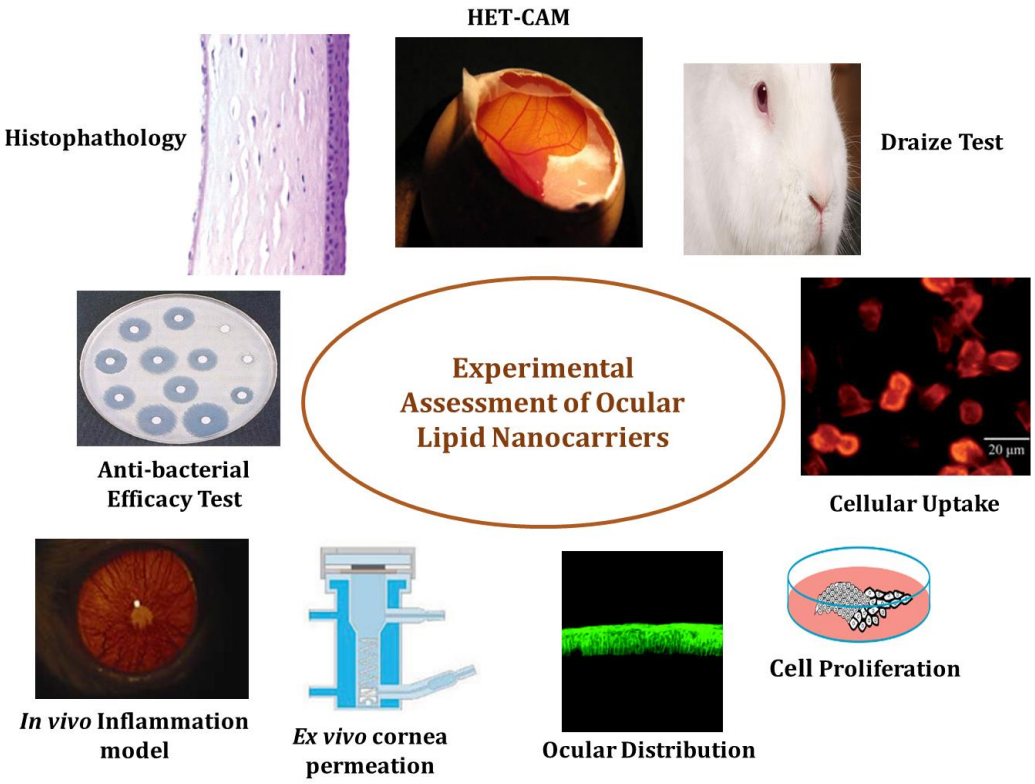

Fig. 5. Illustration presenting the various experiments done to assess the effectiveness and safety of the developed lipid nanocarriers for ocular delivery

6.1. Ocular Safety and Tolerance Study

The cytotoxicity test was often performed on corneal cells, for instance, human corneal epithelial (HCE) and primary human corneal fibroblast (HCFs) cell lines, to determine the ocular safety of the prepared NPs tested compared to non-treated and reference product-

treated cells. Positive cytotoxic control of benzalkonium chloride solution at a concentration of 0.001% could be applied for better comparison. The percent of viable cells was determined relative to control cells using a hemocytometer **[65]** or suitable cytotoxic assays such as MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay **[29]**, **[60]**, **[62]**.

The cell proliferation assay was employed to determine the possible changes in proliferation rate as a tool confirming the ocular tolerability of the tested formulae [29]**.**

The irritation potential of the developed formulae was also tested using hen's egg chorioallantoic membrane (HET-CAM). The egg membrane is examined for any injury, damage, hemorrhage, or coagulation at different times post-application (0.5, 2, and 5 min), and the images were captured. The sum scores for all three irritant responses were calculated and assessed according to a scoring system. Negative and positive controls are treated with a simulated physiological buffer such as normal saline (sodium chloride solution, 0.9%), and a strong irritant such as sodium hydroxide solution (0.5 M) or sodium dodecyl sulfate 10%, respectively **[60]**, **[66]**, **[67]**.

As an *in vivo* experiment, the Draize test was applied to study the potential irritation or damage of the tested formulations on one of the rabbit eyes while the other eye served as a control. After application, any signs of redness, inflammation, and increased lacrimal production are observed and recorded **[68]**.

The histopathological examinations of the animal corneas were also indicative of the safety and tolerability of the applied formulations using stained samples with hematoxylin and eosin and visualized by a light microscope **[65]**. Saline solution and Isopropyl alcohol were used as negative and positive controls, respectively **[69]**.

Moreover, the corneal hydration level was determined to reflect the integrity or damage of the ocular tissue after preparation installation **[69]**. The excised corneas were removed, washed thoroughly, wiped carefully, and then weighed (W_1). These corneas were then subjected to dryness at 50 °C for 24 h and then re-weighed (W_2). The corneal hydration level (%) was then calculated as follows: W1-W2/ W1 × 100 **[69]**.

6.2. Cellular Uptake and Ocular Biodistribution

The uptake of the developed nano-formulations, particularly nanoparticles, into corneal epithelial cells was studied by incubating the tested particles labeled with a fluorescent dye for a certain exposure period. After careful cell washing with phosphate buffer saline (PBS), the intensity of fluorescence is measured using fluorometric analysis or visualized by fluorescence microscopy **[29]**.

The *ex vivo* corneas isolated from animals were used to localize the designated fluorescently-labeled nanoparticles across the corneal epithelium and stroma using fluorescence or confocal laser microscopy (CLSM) **[29]**, **[60]**. Younes *et al.* **[69]** studied the *in vivo* corneal uptake by instilling the Rhodamine B-labeled nanoparticles into the rabbits' eyes, then the eyes were gently washed and the corneas were excised and fixed on glass slides for examination using CLSM.

For *in vivo* ocular disposition and biodistribution, the tested formulations were applied on the animal corneas for the desired regimen, the animals are then anesthetized and euthanized, and their eyes were removed and washed. The different ocular tissues such as the cornea, iris-ciliary body, aqueous and vitreous humor were separated, and the drug amounts in tissue homogenates were determined **[55]**, **[68]**.

6.3. *Ex vivo* Corneal Permeation Experiment

The *ex vivo* drug permeation was often studied after the application of the prepared drug-loaded lipid NPs on excised goat, rabbit, or pig corneas. The corneas are mounted between donor and receptor compartments of Franz diffusion cells. The concentrations of drug permeated across corneas to the simulated tear fluid or 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer containing 133 mM sodium chloride (NaCl, pH 7.4, 32 °C) in the receptor compartment were analyzed using suitable analytical procedures. The cumulative amounts of drug permeated per unit area (Q, µg/cm^2) *versus* time profiles were then constructed, and the steady-state flux (Jss, µg/cm^2.h) was calculated from the slope of the graph linear portion. The permeability coefficient (Kp, cm/h) and enhancement ratio (ER) were also determined as follows: Kp= Jss. Co (Co is the drug concentration in the donor compartment) and ER= Jss of the tested formulation / Jss of the reference product **[60]**, **[66–69]**.

A novel dynamic *ex vivo* permeation study mimicking the natural environment in the eye was executed by de Sá *et al.* **[67]** and illustrated in **Fig. 6**. This was achieved through the excised bovine cornea with simulated tear flow. The cornea was placed into a holder to which a perfusion pump was turned on for flowing simulated tear fluid onto the corneal surface at an adjusted rate (550 µL/min). The anterior and posterior chambers in the corneal holder were filled with the tested formulae and the buffer simulating the physiological medium, respectively. After testing, the corneas were removed, washed, and assayed for corneal opacity using an opacimeter compared to control (untreated) corneas. The amounts of the drug in the excised corneas and the posterior chamber were also analyzed using suitable analytical procedures **[67]**.

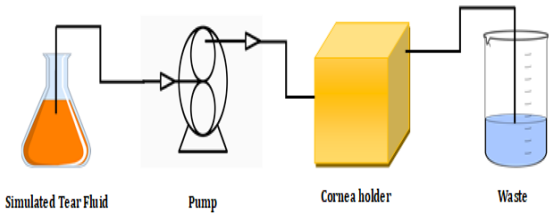

Fig. 6. Diagram showing the dynamic *ex vivo* permeation experiment through excised bovine cornea

6.4. Inflammation Models

The inflammation models were induced either *in vitro* or *in vivo*. In *in vitro* model, HCE cells were stimulated with TNF-α (25 ng/mL) for cellular inflammation, while the *in vivo* inflammation model is induced by treating the animal corneas with lipopolysaccharide (LPS) of *E. Coli* for 24 h. After that, the tested formulations were applied either to cells or viable corneas, and interleukin IL-6 and IL-8 concentrations were then quantified using ELISA (enzyme-linked immunosorbent assay) kit **[29]**, **[60]**, **[62]**.

For endotoxin-induced uveitis, animal eyes were injected with LPS posterior to the limbus in the superotemporal quadrant. After treatment with the formulations understudy, the aqueous humor is aspirated by anterior chamber paracentesis for the determination of leucocyte and prostaglandin E2 (PGE2) levels **[48]**.

6.5. Anti-Bacterial Efficacy Testing

For ocular inflammation caused by bacteria. i.e. bacterial keratitis and conjunctivitis, the anti-bacterial efficiency of the prepared formulations was tested against *Staphylococcus aureus* (*S. aureus)* and *Escherichia coli (E. coli)*. An *in vitro* bacterial culture was incubated with the tested samples and evaluated in comparison to negative controls. The inhibition efficiency was often determined either by analyzing the change in concentration of the bacterial suspension using a UV spectrophotometer at a certain wavelength

and/or by applying the direct-colony counting method **[65]**. The inhibition efficiency (%) was calculated by the following equation:

Inhibition Efficiency (%)= [(Control–treatment)/Control]× 100

The anti-bacterial activity can be also evaluated by the agar cup plate method in which the minimum inhibitory concentrations (MIC) and the diameters of inhibition zones were determined at various time intervals **[66]**.

Induction of ocular conjunctivitis was also executed by instilling 100 microliters (µL) *S. aureus* suspension into the rabbit eyes. After 48 h of bacterial infection, the conjunctivitis is verified by signs of redness, inflammation, and excess lacrimation. The treatment using the tested formulae was started according to the desired regimen. Samples from the treated eyes were then taken by inserting 6-mm filter paper discs into the conjunctival sac of the rabbit eyes. These discs were then incubated in a growth medium and then tested for the density of bacterial growth **[68]**.

Conclusions and Future Perspectives

The treatment of ophthalmic diseases particularly ocular inflammation is a challenge facing scientists and physicians because of the anatomical and physiological barriers of the eye. The topical eye drops and intravitreal injections are the common delivery platforms usually applied in alleviating inflammatory conditions. However, these dosage forms suffer from poor patient compliance being ineffective and unsafe. Nanoparticle drug delivery can be an effective strategy for the transport of therapeutics. Lipid-based nanocarriers can substantially improve drug delivery and can be considered as a promising alternative to conventional therapies. In recent years, various types of lipid platforms are designed and optimized by many researchers mostly for the management of inflammation in both eye segments. This reveals sustainment in drug release and an enhancement in drug bioavailability even to deeper ocular layers and fluids. The inflammatory conditions show a significant improvement with reducing the frequency of administration, and thus increasing patient compliance. In the future, more emphasis has to focus on the effectiveness of lipid nanoparticulate systems in curing the ophthalmic pathologies in the posterior eye segment, this could lead to the development of more innovative lipid platforms for this purpose.

List of Abbreviations

ACV, Acyclovir; AmB, Amphotericin B; AUC, Area under the curve; BAV, Bioavailability; $CaCl_2$, Calcium Chloride; CH, Chitosan oligosaccharide; COL, Chitosan oligosaccharide lactate; Cmax, Maximum drug concentration; DEX, Dexamethasone; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; EE, Entrapment Efficiency; HET-CAM: hen's egg-chorioallantoic membrane; HPMC, Hydroxypropyl methyl cellulose; KT, Ketorolac tromethamine; LXN, Lomefloxacin HCl; ME, Microemulsion; MOX, Moxifloxacin; MIC, Minimum inhibitory concentration; mPEG-2K-DSPE:1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine with conjugated methoxyl poly(ethylene glycol) 2000; MOX: Moxifloxacin; NaOH, Sodium hydroxide; NAT, Natamycin; NE, Nanoemulsion; NPs, Nanoparticles; NLC, Nanostructured lipid carrier; OFX: Ofloxacin; PC: Soy Phosphatidylcholine; PDI: Polydispersity index; PEG: Polyethylene glycol; PS: Particle size; PSED: Posterior segment eye disease; RI: Refractive index; SA, Stearic acid; SLN, solid lipid nanoparticles; STZ, Sertaconazole nitrate; Tmax, Time at maximum drug concentration; VOR, voriconazole; ZP, Zeta potential; 3D HCE-T model, 3D human corneal epithelial tissue model.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All literature is available

Competing interests

The authors report no conflicts of interest.

Funding statement

No funding source was received

Authors' contributions

Doaa Hamdy: collecting literature, and writing the first draft of the review.

Rania A.H. Ishak, Amany O. Kamel, and Nahed D. Mortada: organizing, editing, and reviewing the manuscript.

All authors read and approved the final manuscript.

7. REFERENCES

1. D. K. Sigford, S. Reddy, C. Mollineaux, and S. Schaal, "Global reported endophthalmitis risk following intravitreal injections of anti-VEGF: A literature review and analysis," *Clin. Ophthalmol.*, vol. 9, pp. 773–781, May 2015, doi: 10.2147/OPTH.S77067.

2. M. L. Occhiutto, F. R. Freitas, R. C. Maranhao, and V. P. Costa, "Breakdown of the blood-ocular barrier as a strategy for the systemic use of nanosystems," *Pharmaceutics*, vol. 4, no. 2. Pharmaceutics, pp. 252–275, Jun. 2012, doi: 10.3390/pharmaceutics4020252.

3. V. Agrahari *et al.*, "A comprehensive insight on ocular pharmacokinetics," *Drug Delivery and Translational Research*, vol. 6, no. 6. Springer Verlag, pp. 735–754, Dec. 01, 2016, doi: 10.1007/s13346-016-0339-2.

4. E. Ramsay *et al.*, "Corneal and conjunctival drug permeability: Systematic comparison and pharmacokinetic impact in the eye," *Eur. J. Pharm. Sci.*, vol. 119, pp. 83–89, Jul. 2018, doi: 10.1016/j.ejps.2018.03.034.

5. V. Citi, E. Peragine, S. Brogi, S. Ottino, and V. Calderone, "Development of In Vitro Corneal Models: Opportunity for Pharmacological Testing.," *Methods Protoc.*, vol. 3, no. 4, pp. 1–12, Nov. 2020, doi: 10.3390/mps3040074.

6. M. S. Sridhar, "Anatomy of the cornea and ocular surface," *Indian Journal of Ophthalmology*, vol. 66, no. 2. Medknow Publications, pp. 190–194, Feb. 01, 2018, doi: 10.4103/ijo.IJO_646_17.

7. C. R. Lynch, P. P. D. Kondiah, Y. E. Choonara, L. C. du Toit, N. Ally, and V. Pillay, "Hydrogel Biomaterials for Application in Ocular Drug Delivery," *Frontiers in Bioengineering and Biotechnology*, vol. 8. Frontiers Media S.A., Mar. 20, 2020, doi: 10.3389/fbioe.2020.00228.

8. C. L. Shumway, M. Motlagh, and M. Wade, "Anatomy, Head and Neck, Eye Conjunctiva.," Treasure Island (FL), 2021.

9. B. A. Schechter, "Use of topical bromfenac for treating ocular pain and inflammation beyond cataract surgery: A review of published studies," *Clinical Ophthalmology*, vol. 13. Dove Medical Press Ltd, pp. 1439–1460, 2019, doi: 10.2147/OPTH.S208700.

10. B. I. Gaynes and R. Fiscella, "Topical nonsteroidal anti-inflammatory drugs for ophthalmic use: A safety review," *Drug Safety*, vol. 25, no. 4. Adis International Ltd, pp. 233–250, 2002, doi: 10.2165/00002018-200225040-00002.

11. B. Yavuz and U. B. Kompella, "Ocular drug

delivery," in *Handbook of Experimental Pharmacology*, vol. 242, Springer New York LLC, 2017, pp. 57–93.

12. J. B. Naik, S. R. Pardeshi, R. P. Patil, P. B. Patil, and A. Mujumdar, "Mucoadhesive Micro-/Nano Carriers in Ophthalmic Drug Delivery: an Overview," *BioNanoScience*, vol. 10, no. 3. Springer, pp. 564–582, Sep. 01, 2020, doi: 10.1007/s12668-020-00752-y.

13. R. Mazet, J. B. G. G. Yaméogo, D. Wouessidjewe, L. Choisnard, and A. Gèze, *No Title*, vol. 12, no. 6. MDPI AG, 2020, pp. 1–56.

14. L. L. Tundisi, G. B. Mostaço, P. C. Carricondo, and D. F. S. Petri, "Hydroxypropyl methylcellulose: Physicochemical properties and ocular drug delivery formulations," *Eur. J. Pharm. Sci.*, vol. 159, Apr. 2021, doi: 10.1016/j.ejps.2021.105736.

15. T. Irimia, M. V. Ghica, L. Popa, V. Anuţa, A. L. Arsene, and C. E. Dinu-Pîrvu, "Strategies for improving ocular drug bioavailability and corneal wound healing with chitosan-based delivery systems," *Polymers*, vol. 10, no. 11. MDPI AG, Nov. 03, 2018, doi: 10.3390/polym10111221.

16. N. Dubashynskaya, D. Poshina, S. Raik, A. Urtti, and Y. A. Skorik, "Polysaccharides in ocular drug delivery," *Pharmaceutics*, vol. 12, no. 1. MDPI AG, Jan. 01, 2020, doi: 10.3390/pharmaceutics12010022.

17. G. M. Grass and J. R. Robinson, "Relationship of chemical structure to corneal penetration and influence of low-viscosity solution on ocular bioavailability," *J. Pharm. Sci.*, vol. 73, no. 8, pp. 1021–1027, 1984, doi: 10.1002/jps.2600730803.

18. A. K. Agrawal, M. Das, and S. Jain, "In situ gel systems as 'smart' carriers for sustained ocular drug delivery," *Expert Opinion on Drug Delivery*, vol. 9, no. 4. Expert Opin Drug Deliv, pp. 383–402, Apr. 2012, doi: 10.1517/17425247.2012.665367.

19. A. A. A. Youssef, C. Cai, N. Dudhipala, and S. Majumdar, "Design of Topical Ocular Ciprofloxacin Nanoemulsion for the Management of Bacterial Keratitis.," *Pharmaceuticals (Basel).*, vol. 14, no. 3, Mar. 2021, doi: 10.3390/ph14030210.

20. A. Youssef, N. Dudhipala, and S. Majumdar, "Ciprofloxacin Loaded Nanostructured Lipid Carriers Incorporated into In-Situ Gels to Improve Management of Bacterial Endophthalmitis.," *Pharmaceutics*, vol. 12, no. 6, pp. 1–19, Jun. 2020, doi: 10.3390/pharmaceutics12060572.

21. M. H. Abraham and A. A. Mahmoud, "Biodegradable ocular inserts for sustained delivery of brimonidine tartrate: Preparation and in vitro/in vivo evaluation," *AAPS PharmSciTech*, vol. 12, no. 4, pp. 1335–1347, Dec. 2011, doi: 10.1208/s12249-011-9701-3.

22. M. Lengyel, N. Kállai-Szabó, V. Antal, A. J. Laki, and I. Antal, "Microparticles, microspheres, and microcapsules for advanced drug delivery," *Scientia Pharmaceutica*, vol. 87, no. 3. MDPI AG, p. 20, Sep. 01, 2019, doi: 10.3390/scipharm87030020.

23. R. H. Müller, K. Mäder, and S. Gohla, "Solid lipid nanoparticles (SLN) for controlled drug delivery - A review of the state of the art," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 50, no. 1. Eur J Pharm Biopharm, pp. 161–177, Jul. 03, 2000, doi: 10.1016/S0939-6411(00)00087-4.

24. D. R. Janagam, L. Wu, and T. L. Lowe, "Nanoparticles for drug delivery to the anterior segment of the eye," *Advanced Drug Delivery Reviews*, vol. 122. Elsevier B.V., pp.

31–64, Dec. 01, 2017, doi: 10.1016/j.addr.2017.04.001.

25. A. Akbarzadeh *et al.*, “Liposome: Classification, preparation, and applications,” *Nanoscale Res. Lett.*, vol. 8, no. 1, 2013, doi: 10.1186/1556-276X-8-102.

26. L. Maja, K. Željko, and P. Mateja, “Sustainable technologies for liposome preparation,” *Journal of Supercritical Fluids*, vol. 165. Elsevier B.V., p. 104984, Nov. 01, 2020, doi: 10.1016/j.supflu.2020.104984.

27. N. S. Elbialy, B. M. Abdol-Azim, M. W. Shafaa, L. H. El Shazly, A. H. El Shazly, and W. A. Khalil, “Enhancement of the ocular therapeutic effect of prednisolone acetate by liposomal entrapment,” *J. Biomed. Nanotechnol.*, vol. 9, no. 12, pp. 2105–2116, Dec. 2013, doi: 10.1166/jbn.2013.1711.

28. H. F. Salem, S. M. Ahmed, and M. M. Omar, “Liposomal flucytosine capped with gold nanoparticle formulations for improved ocular delivery,” *Drug Des. Devel. Ther.*, vol. 10, pp. 277–295, Jan. 2016, doi: 10.2147/DDDT.S91730.

29. L. Soriano-Romaní *et al.*, “Novel anti-inflammatory liposomal formulation for the pre-ocular tear film: In vitro and ex vivo functionality studies in corneal epithelial cells,” *Exp. Eye Res.*, vol. 154, pp. 79–87, Jan. 2017, doi: 10.1016/j.exer.2016.11.010.

30. M. Feghhi, B. Sharif Makhmalzadeh, F. Farrahi, M. Akmal, and N. Hasanvand, "Anti-microbial Effect and in Vivo Ocular Delivery of Ciprofloxacin-loaded Liposome through Rabbit's Eye," *Curr. Eye Res.*, vol. 45, no. 10, pp. 1245–1251, Oct. 2020, doi: 10.1080/02713683.2020.1728777.

31. R. Suri, Y. R. Neupane, N. Mehra, G. K. Jain, and K. Kohli, “Sirolimus loaded polyol modified liposomes for the treatment of Posterior Segment Eye Diseases,” *Med. Hypotheses*, vol. 136, Mar. 2020, doi: 10.1016/j.mehy.2019.109518.

32. I. F. Uchegbu and S. P. Vyas, “Non-ionic surfactant based vesicles (niosomes) in drug delivery,” *Int. J. Pharm.*, vol. 172, no. 1–2, pp. 33–70, Oct. 1998, doi: 10.1016/S0378-5173(98)00169-0.

33. B. W. Barry, “Novel mechanisms and devices to enable successful transdermal drug delivery,” *European Journal of Pharmaceutical Sciences*, vol. 14, no. 2. Eur J Pharm Sci, pp. 101–114, 2001, doi: 10.1016/S0928-0987(01)00167-1.

34. C. Marianecci *et al.*, "Niosomes from the 80s to present: The state of the art," *Advances in Colloid and Interface Science*, vol. 205. pp. 187–206, Mar. 2014, doi: 10.1016/j.cis.2013.11.018.

35. P. M. E. Gaafar, O. Y. Abdallah, R. M. Farid, and H. Abdelkader, "Preparation, characterization, and evaluation of novel elastic nano-sized niosomes (ethoniosomes) for ocular delivery of prednisolone," *J. Liposome Res.*, vol. 24, no. 3, pp. 204–215, 2014, doi: 10.3109/08982104.2014.881850.

36. O. Ashraf, M. Nasr, M. Nebsen, A. M. A. Said, and O. Sammour, “In vitro stabilization and in vivo improvement of ocular pharmacokinetics of the multi-therapeutic agent baicalin: Delineating the most suitable vesicular systems,” *Int. J. Pharm.*, vol. 539, no. 1–2, pp. 83–94, Mar. 2018, doi: 10.1016/j.ijpharm.2018.01.041.

37. R. Kapadia, K. Parikh, M. Jain, and K. Sawant, "Topical instillation of triamcinolone acetonide-loaded emulsions for posterior ocular delivery: statistical optimization and in vitro-in vivo studies," *Drug Deliv. Transl. Res.*, vol. 11, no. 3, pp. 984–999, Jun. 2021, doi: 10.1007/s13346-020-00810-8.

38. Z. Karami and M. Hamidi, “Cubosomes: Remarkable drug delivery potential,” *Drug Discovery Today*, vol. 21, no. 5. Elsevier Ltd, pp. 789–801, May 01, 2016, doi: 10.1016/j.drudis.2016.01.004.

39. I. D. M. Azmi *et al.*, "Cisplatin Encapsulation Generates Morphologically Different Multi compartments in the Internal Nanostructures of Nonlamellar Liquid-Crystalline Self-Assemblies," *Langmuir*, vol. 34, no. 22, pp. 6570–6581, Jun. 2018, doi: 10.1021/acs.Langmuir.8b01149.

40. G. Garg, S. Saraf, and S. Saraf, “Cubosomes: An overview,” *Biological and Pharmaceutical Bulletin*, vol. 30, no. 2. Biol Pharm Bull, pp. 350–353, Feb. 2007, doi: 10.1248/bpb.30.350.

41. M. A. El-Gendy, M. Mansour, M. I. A. El-Assal, R. A. H. Ishak, and N. D. Mortada, “Delineating penetration enhancer-enriched liquid crystalline nanostructures as novel platforms for improved ophthalmic delivery,” *Int. J. Pharm.*, vol. 582, May 2020, doi: 10.1016/j.ijpharm.2020.119313.

42. M. A. Elfaky *et al.*, “Development, Optimization, and Antifungal Assessment of Ocular Gel Loaded With Ketoconazole Cubic Liquid Crystalline Nanoparticles,” *J. Pharm. Sci.*, vol. 110, no. 5, pp. 2210–2220, May 2021, doi: 10.1016/j.xphs.2021.02.022.

43. C. D. V. Bessone, S. P. Akhlaghi, L. I. Tártara, D. A. Quinteros, W. Loh, and D. A. Allemandi, “Latanoprost-loaded phytantriol cubosomes for the treatment of glaucoma,” *Eur. J. Pharm. Sci.*, vol. 160, May 2021, doi: 10.1016/j.ejps.2021.105748.

44. J. Huang *et al.*, “Ocular Cubosome Drug Delivery System for Timolol Maleate: Preparation, Characterization, Cytotoxicity, Ex Vivo, and In Vivo Evaluation,” *AAPS PharmSciTech*, vol. 18, no. 8, pp. 2919–2926, Nov. 2017, doi: 10.1208/s12249-017-0763-8.

45. S. A. Gaballa, O. H. El Garhy, H. Moharram, and H. Abdelkader, “Preparation and Evaluation of Cubosomes/Cubosomal Gels for Ocular Delivery of Beclomethasone Dipropionate for Management of Uveitis,” *Pharm. Res.*, vol. 37, no. 10, Oct. 2020, doi: 10.1007/s11095-020-02857-1.

46. N. Anton, J. P. Benoit, and P. Saulnier, “Design and production of nanoparticles formulated from nano-emulsion templates-A review,” *Journal of Controlled Release*, vol. 128, no. 3. J Control Release, pp. 185–199, Jun. 24, 2008, doi: 10.1016/j.jconrel.2008.02.007.

47. S. S. Smail, M. M. Ghareeb, H. K. Omer, A. A. Al-Kinani, and R. G. Alany, “Studies on Surfactants, Cosurfactants, and Oils for Prospective Use in Formulation of Ketorolac Tromethamine Ophthalmic Nanoemulsions.,” *Pharmaceutics*, vol. 13, no. 4, Mar. 2021, doi: 10.3390/pharmaceutics13040467.

48. J. Shen *et al.*, “Novel NSAIDs ophthalmic formulation: Flurbiprofen axetil emulsion with low irritancy and improved anti-inflammation effect,” *Int. J. Pharm.*, vol. 412, no. 1–2, pp. 115–122, Jun. 2011, doi: 10.1016/j.ijpharm.2011.03.041.

49. S. Ligório Fialho and A. da Silva-Cunha, “New vehicle based on a microemulsion for topical ocular administration of dexamethasone,” *Clin. Exp. Ophthalmol.*, vol. 32, no. 6, pp. 626–632, Dec. 2004, doi: 10.1111/j.1442-9071.2004.00914.x.

50. K. Kesavan, S. Kant, P. N. Singh, and J. K. Pandit, “Mucoadhesive chitosan-coated cationic microemulsion of dexamethasone for ocular delivery: In vitro and in vivo evaluation,” *Curr. Eye Res.*, vol. 38, no. 3, pp. 342–352, Mar. 2013, doi:

10.3109/02713683.2012.745879.

51. A. Mahran, S. Ismail, and A. A. Allam, “Development of Triamcinolone Acetonide-Loaded Microemulsion as a Prospective Ophthalmic Delivery System for Treatment of Uveitis: In Vitro and In Vivo Evaluation.,” *Pharmaceutics*, vol. 13, no. 4, Mar. 2021, doi: 10.3390/pharmaceutics13040444.

52. W. Mehnert and K. Mäder, “Solid lipid nanoparticles: Production, characterization and applications,” *Adv. Drug Deliv. Rev.*, vol. 47, no. 2–3, pp. 165–196, Apr. 2001, doi: 10.1016/S0169-409X(01)00105-3.

53. V. A. Duong, T. T. L. Nguyen, and H. J. Maeng, “Preparation of solid lipid nanoparticles and nanostructured lipid carriers for drug delivery and the effects of preparation parameters of solvent injection method,” *Molecules*, vol. 25, no. 20. MDPI AG, Oct. 01, 2020, doi: 10.3390/molecules25204781.

54. A. K. Sharma, P. K. Sahoo, D. K. Majumdar, N. Sharma, R. K. Sharma, and A. Kumar, "Fabrication and evaluation of lipid nanoparticles for ocular delivery of a COX-2 inhibitor," *Drug Deliv.*, vol. 23, no. 9, pp. 3364–3373, Nov. 2016, doi: 10.1080/10717544.2016.1183720.

55. A. Take *et al.*, “In situ gel of triamcinolone acetonide-loaded solid lipid nanoparticles for improved topical ocular delivery: Tear kinetics and ocular disposition studies,” *Nanomaterials*, vol. 9, no. 1, Jan. 2019, doi: 10.3390/nano9010033.

56. S. Safwat, R. A. H. Ishak, R. M. Hathout, and N. D. Mortada, “Nanostructured lipid carriers loaded with simvastatin: effect of PEG/glycerides on characterization, stability, cellular uptake efficiency and in vitro cytotoxicity,” *Drug Dev. Ind. Pharm.*, vol. 43, no. 7, 2017, doi: 10.1080/03639045.2017.1293681.

57. L. Battaglia *et al.*, “Solid lipid nanoparticles for potential doxorubicin delivery in glioblastoma treatment: Preliminary in vitro studies,” *J. Pharm. Sci.*, vol. 103, no. 7, pp. 2157–2165, 2014, doi: 10.1002/jps.24002.

58. S. Gade *et al.*, “An Ex Vivo Evaluation of Moxifloxacin Nanostructured Lipid Carrier Enriched In Situ Gel for Transcorneal Permeation on Goat Cornea,” *J. Pharm. Sci.*, vol. 108, no. 9, pp. 2905–2916, Sep. 2019, doi: 10.1016/j.xphs.2019.04.005.

59. F. Zahir-Jouzdani *et al.*, “Nanostructured lipid carriers containing rapamycin for prevention of corneal fibroblasts proliferation and haze propagation after burn injuries: In vitro and in vivo,” *J. Cell. Physiol.*, vol. 234, no. 4, pp. 4702–4712, Apr. 2019, doi: 10.1002/jcp.27243.

60. P. Nirbhavane *et al.*, "Triamcinolone acetonide loaded-cationic nano-lipoidal formulation for uveitis: Evidence of improved biopharmaceutical performance and anti-inflammatory activity," *Colloids Surfaces B Biointerfaces*, vol. 190, Jun. 2020, doi: 10.1016/j.colsurfb.2020.110902.

61. S. Safwat, R. M. Hathout, R. A. Ishak, and N. D. Mortada, “Augmented simvastatin cytotoxicity using optimized lipid nanocapsules: a potential for breast cancer treatment,” *J. Liposome Res.*, 2016, doi: 10.3109/08982104.2015.1137313.

62. M. L. Formica, G. V. Ullio Gamboa, L. I. Tártara, J. D. Luna, J. P. Benoit, and S. D. Palma, “Triamcinolone acetonide-loaded lipid nanocapsules for ophthalmic applications,” *Int. J. Pharm.*, vol. 573, Jan. 2020, doi: 10.1016/j.ijpharm.2019.118795.

63. M. L. Formica *et al.*, “Novel hybrid lipid nanocapsules loaded with a therapeutic monoclonal antibody – Bevacizumab – and

Triamcinolone acetonide for combined therapy in neovascular ocular pathologies," *Mater. Sci. Eng. C*, vol. 119, Feb. 2021, doi: 10.1016/j.msec.2020.111398.

64. L. C. Du Toit, T. Govender, T. Carmichael, P. Kumar, Y. E. Choonara, and V. Pillay, "Design of an anti-inflammatory composite nanosystem and evaluation of its potential for ocular drug delivery," *J. Pharm. Sci.*, vol. 102, no. 8, pp. 2780–2805, 2013, doi: 10.1002/jps.23650.

65. M. C. Chang, Y. J. Kuo, K. H. Hung, C. L. Peng, K. Y. Chen, and L. K. Yeh, "Liposomal dexamethasone-moxifloxacin nanoparticle combinations with collagen/gelatin/alginate hydrogel for corneal infection treatment and wound healing," *Biomed. Mater.*, vol. 15, no. 5, Sep. 2020, doi: 10.1088/1748-605X/ab9510.

66. M. S. Baig, A. Ahad, M. Aslam, S. S. Imam, M. Aqil, and A. Ali, "Application of Box-Behnken design for preparation of levofloxacin-loaded stearic acid solid lipid nanoparticles for ocular delivery: Optimization, in vitro release, ocular tolerance, and antibacterial activity," *Int. J. Biol. Macromol.*, vol. 85, pp. 258–270, Apr. 2016, doi: 10.1016/j.ijbiomac.2015.12.077.

67. F. A. P. de Sá, S. F. Taveira, G. M. Gelfuso, E. M. Lima, and T. Gratieri, "Liposomal voriconazole (VOR) formulation for improved ocular delivery," *Colloids Surfaces B Biointerfaces*, vol. 133, pp. 331–338, Sep. 2015, doi: 10.1016/j.colsurfb.2015.06.036.

68. A. Patil *et al.*, "Formulation Development, Optimization, and In Vitro–In Vivo Characterization of Natamycin-Loaded PEGylated Nano-Lipid Carriers for Ocular Applications," *J. Pharm. Sci.*, vol. 107, no. 8, pp. 2160–2171, Aug. 2018, doi: 10.1016/j.xphs.2018.04.014.

69. N. F. Younes, S. A. Abdel-Halim, and A. I. Elassasy, "Corneal targeted Sertaconazole nitrate loaded cubosomes: Preparation, statistical optimization, in vitro characterization, ex vivo permeation and in vivo studies," *Int. J. Pharm.*, vol. 553, no. 1–2, pp. 386–397, Dec. 2018, doi: 10.1016/j.ijpharm.2018.10.057.

70. S. A. Tayel, M. A. El-Nabarawi, M. I. Tadros, and W. H. Abd-Elsalam, "Promising ion-sensitive in situ ocular nanoemulsion gels of terbinafine hydrochloride: Design, in vitro characterization and in vivo estimation of the ocular irritation and drug pharmacokinetics in the aqueous humor of rabbits," *Int. J. Pharm.*, vol. 443, no. 1–2, pp. 293–305, Feb. 2013, doi: 10.1016/j.ijpharm.2012.12.049.

71. B. Jurišić Dukovski *et al.*, "Functional ibuprofen-loaded cationic nanoemulsion: Development and optimization for dry eye disease treatment," *Int. J. Pharm.*, vol. 576, Feb. 2020, doi: 10.1016/j.ijpharm.2019.118979.

72. N. Üstündag-Okur, E. H. Gökçe, S. Eğrilmez, Ö. Özer, and G. Ertan, "Novel ofloxacin-loaded microemulsion formulations for ocular delivery," *J. Ocul. Pharmacol. Ther.*, vol. 30, no. 4, pp. 319–332, May 2014, doi: 10.1089/jop.2013.0114.

73. S. K. Bharti and K. Kesavan, "Phase-transition W/O Microemulsions for Ocular Delivery: Evaluation of Antibacterial Activity in the Treatment of Bacterial Keratitis," *Ocul. Immunol. Inflamm.*, vol. 25, no. 4, pp. 463–474, Jul. 2017, doi: 10.3109/09273948.2016.1139136.

74. R. M. Khalil, G. A. Abdelbary, M. Basha, G. E. A. Awad, and H. A. el-Hashemy, "Enhancement of lomefloxacin Hcl ocular efficacy via liposomal encapsulation: in vitro characterization and in vivo evaluation," *J.*

Liposome Res., vol. 27, no. 4, pp. 312–323, Oct. 2017, doi: 10.1080/08982104.2016.1191022.

75. O. A. E. A. Soliman, E. A. Mohamed, and N. A. A. Khatera, "Enhanced ocular bioavailability of fluconazole from liposomal gels and microemulsions: formulation, optimization, and in vitro–in vivo evaluation," *Pharm. Dev. Technol.*, vol. 24, no. 1, pp. 48–62, Jan. 2019, doi: 10.1080/10837450.2017.1413658.

76. M. A. El-Nabarawi *et al.*, "Natamycin niosomes as a promising ocular nanosized delivery system with ketorolac tromethamine for dual effects for the treatment of candida rabbit keratitis; in vitro/in vivo and histopathological studies," *Drug Dev. Ind. Pharm.*, vol. 45, no. 6, pp. 922–936, Jun. 2019, doi: 10.1080/03639045.2019.1579827.

77. R. M. Khalil, G. A. Abdelbary, M. Basha, G. E. A. Awad, and H. A. El-Hashemy, "Design and evaluation of proteasomes as a carrier for ocular delivery of lomefloxacin HCl," *J. Liposome Res.*, vol. 27, no. 2, pp. 118–129, Apr. 2017, doi: 10.3109/08982104.2016.1167737.

78. L. Arana *et al.*, “Solid lipid nanoparticles for delivery of Calendula officinalis extract,” *Colloids Surfaces B Biointerfaces*, vol. 135, pp. 18–26, Nov. 2015, doi: 10.1016/j.colsurfb.2015.07.020.

79. N. Üstündağ-Okur, E. H. Gökçe, D. I. Bozbiyik, S. Eğrilmez, Ö. Özer, and G. Ertan, "Preparation and in vitro-in vivo evaluation of ofloxacin loaded ophthalmic nanostructured lipid carriers modified with chitosan oligosaccharide lactate for the treatment of bacterial keratitis," *Eur. J. Pharm. Sci.*, vol. 63, pp. 204–215, Oct. 2014, doi: 10.1016/j.ejps.2014.07.013.

80. N. Üstündağ-Okur, E. H. Gökçe, D. I. Bozbiyik, S. Eğrilmez, G. Ertan, and Ö. Özer, “Novel nanostructured lipid carrier-based inserts for controlled ocular drug delivery: Evaluation of corneal bioavailability and treatment efficacy in bacterial keratitis,” *Expert Opinion on Drug Delivery*, vol. 12, no. 11. Taylor and Francis Ltd, pp. 1791–1807, Nov. 02, 2015, doi: 10.1517/17425247.2015.1059419.

81. A. Seyfoddin *et al.*, “Ex vivo and In vivo Evaluation of Chitosan Coated Nanostructured Lipid Carriers for Ocular Delivery of Acyclovir,” *Curr. Drug Deliv.*, vol. 13, no. 6, pp. 923–934, Aug. 2016, doi: 10.2174/1567201813666151116142752.

82. T. Fu, J. Yi, S. Lv, and B. Zhang, “Ocular amphotericin B delivery by chitosan-modified nanostructured lipid carriers for fungal keratitis-targeted therapy,” *J. Liposome Res.*, vol. 27, no. 3, pp. 228–233, Jul. 2017, doi: 10.1080/08982104.2016.1224899.

83. N. Tavakoli, S. Taymouri, A. Saeidi, and V. Akbari, "Thermosensitive hydrogel containing sertaconazole loaded nanostructured lipid carriers for the potential treatment of fungal keratitis," *Pharm. Dev. Technol.*, vol. 24, no. 7, pp. 891–901, Aug. 2019, doi: 10.1080/10837450.2019.1616755.