



# Innovative niosomal *in-situ* gel: Elevating ocular drug delivery synergies

Neha Kandpal, Rakshanda Dhuliya, Neha Padiyar, Abhishek Singh, Shivansh Khadiyal, Yogita Ale, Vikash Jakhmola, Nidhi Nainwal\* 

Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India.

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## ABSTRACT

The eye is an intricate organ with multiple defense mechanisms and protective barriers. This organ is susceptible to infections, hereditary abnormalities, and vision impairments. Therefore, it is necessary to administer medicine to the eyes through the appropriate method. The conventional approach of ocular drug administration may be inefficient due to limited bioavailability. Targeted drug delivery systems based on nanocarriers can overcome some restrictions encountered due to the complex structure of the eye. *In situ*, gel-loaded niosomes may offer advantages in the field of ocular drug delivery. Niosomes, act as a promising carrier for hydrophobic and hydrophilic medicines, shielding them from fast clearance and degradation and allowing persistent release in ocular tissues. The inclusion of niosomes in the *in-situ* gels provides increased corneal retention, thereby ensuring enhanced medication penetration and prolonged contact time. Furthermore, niosomes are a potential choice for long-term therapy because of their ability to offer sustained drug release, lower dose frequency, and minimize systemic side effects. This article presents a detailed review of the current state of research on niosomes-loaded *in-situ* gel for ocular distribution, focusing on formulation techniques, characterization, toxicity, mechanisms of action, mechanisms of sol-gel transition, and prospective uses in various ocular illness. In-depth reviews of the various corneal penetration and absorption models for the *in-vitro*, *in-vivo*, and *ex vivo* research are also presented, along with a summary of the various patents and the commercial formulation of *in-situ* gels.

## INTRODUCTION

A colloidal drug delivery system is an innovative approach in the field of pharmaceuticals and nanotechnology that aims to enhance the therapeutic effectiveness of drugs. It involves the utilization of colloidal particles, typically ranging in size from 1 to 1,000 nanometers, to encapsulate and deliver drugs to specific target sites within the body [1]. This technology has gained considerable attention due to its ability to overcome many challenges associated with traditional drug delivery methods. A colloidal drug delivery system includes liposomes, niosomes, micelles, dendrimers, *in-situ* gels, and so on [2–5]. It provides significant advancements in the field of ocular drug delivery, offering promising

solutions (Sols) for treating various ocular disorders by overcoming numerous barriers such as corneal, precorneal, conjunctival, and formulation challenges [6]. Niosomes have grown in prominence as the most effective drug carriers in ocular therapies. The small vesicle size of niosomes and their inability to permeate connective tissue and epithelium deliver the drug at the administration site [7].

However, niosomes suffer from poor precorneal retention and nasolacrimal drainage [8]. Therefore, to overcome this problem, niosomes are being incorporated into an *in-situ* gel [9]. A system known as “*in-situ* gel” is one that, when exposed to physiological circumstances such as variations in temperature, pH, or ion concentration, goes through a phase transition from a Sol or liquid state to a gel or semi-solid state. These systems provide controlled and sustained release of drugs for an extended period. *In situ*, gel formulations are predominantly based on biocompatible polymers, such as thermosensitive polymers, mucoadhesive polymers, and biodegradable hydrogels, which

\*Corresponding Author  
Nidhi Nainwal, Uttaranchal Institute of Pharmaceutical Sciences,  
Uttaranchal University, Dehradun, India.  
E-mail: [nidhi.nainwal87@gmail.com](mailto:nidhi.nainwal87@gmail.com)

provide the necessary mechanical strength, stability, and biodegradability [10].

The potential for a paradigm shift in ocular treatments occurs with the combination of colloidal and *in-situ* delivery systems which overcome physiological and anatomical limitations of ocular delivery [11]. In terms of improving solubility, stability, targeting, prolonged release, and adaptability, colloidal drug delivery methods are a promising new direction for the pharmaceutical industry. This current review provides an overview of combining *in situ* gel with niosomes for ocular delivery of many therapeutic agents. An in-depth review has been made focusing on various formulation, characterization, safety, and development prospects of *in situ* gels loaded with niosomes for ocular administration.

Niosomes have small vesicle sizes and allow limited penetration into the epithelium and connective tissue, keeping the medication at the administration site. However, precorneal and nasolacrimal drainage limit the applicability of niosomes in ocular delivery because of their low viscosity. The inclusion of niosomes in *in-situ* gels ensures prolonged retention on the ocular surface, resulting in improved medicine penetration and extended contact. Furthermore, because of their ability to minimize systemic side effects, lower dose frequency, and delayed drug release, make them potential candidates for long-term therapies [12]. Continued research and development in this field hold great potential for optimizing drug therapies and improving patient outcomes across various medical conditions.

## **NIOSOMES IN OCULAR DRUG DELIVERY**

The eyes are the key sensory organ responsible for vision. In the skull, the eyes are in bony spaces known as orbits. Its shape is roughly spherical, with one of its inner layers—the cornea and sclera make up the outermost layer, also referred to as the fibrous tunic [13]. The choroid, ciliary body, pigmented epithelium, and iris make up the middle layer, also referred to as the vascular tunic or uvea. The retina is the innermost layer. Retinal blood arteries (anteriorly) and choroid blood vessels (posteriorly) supply oxygen to the retina. The vitreous body, a jelly-like material, fills the entire posterior chamber of the eye, whereas the aqueous humor fills the area anteriorly, between the cornea and lens. Conjunctiva, a thin clear layer lies on top of this [14].

Ocular drug delivery is a challenging field due to the presence of various static and dynamic barriers present in the eye. Static barriers include nasolacrimal drainage, blinking, cornea, sclera, and blood-queous barriers, whereas dynamic barriers include conjunctival blood flow, lymphatic clearing, and tear drainage [13,6]. Overcoming the ocular barrier using nanotechnology holds a revolutionizing approach for the treatment of a variety of eye disorders [15]. Nanotechnology plays a significant role in ocular drug delivery through nanoparticles, niosomes, liposomes, micelles, dendrimers, and other polymeric vesicles. Due to their affordability and stability in Sols and during storage niosomes are becoming more and more popular as drug-delivery nanocarriers [16]. These are non-ionic surfactant vesicles that can encapsulate drugs and protect them from degradation while facilitating their transport across ocular

barriers [17]. Small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles are the three categories into which niosomes are divided according to their size or number of bilayers. These non-ionic vesicles have particles that are submicron in size. Commonly employed methods for formulating niosomes are the thin film hydration method, reverse phase evaporation (REV), ether injection method, handshaking method, and hydration by freezing and thawing [17]. Drug loading in niosomes is a crucial step in formulating niosomes for targeted and controlled delivery. Proper drug loading ensures the efficient delivery of drugs to the target site [18]. Active loading and passive loading are some drug loading techniques [19]. Passive drug loading involves passively encapsulating the drug inside the niosomal vesicles during the hydration process. Active loading involves encapsulating medicinal molecules into niosomes using a transmembrane gradient [20]. The drug diffuses across the niosome membrane and builds up inside the niosome through protonation. The primary benefit of this approach over passive loading techniques is its high drug loading efficiency [21].

## **IN-SITU GELLING SYSTEM**

*In situ*, gelling formulations are drug delivery systems that are generally liquid at ambient temperature but convert to gel after being applied to the body in response to numerous stimuli such as temperature, pH, and ionic composition. The term “*in situ* gel-forming systems” refers to low-viscosity Sols that, in reaction to the physiological environment, experience conformational changes in polymers and undergo a phase transition in the conjunctival *cul-de-sac* to create viscoelastic gels [10]. *In situ* gels can be made using both natural and synthetic polymers. Gelation imparts new viscoelastic properties, restricting pre-corneal removal and resulting in a longer residence time on the ocular surface [22].

## **MECHANISMS OF SOL-GEL FORMULATION**

*In situ*, gels undergo a phase transition from a liquid/sol state to a gel state upon specific trigger or environmental conditions. Mechanisms of sol-gel formation for niosomal *in-situ* gel can vary depending on the specific formulation and the stimuli used. The most common and briefly described method for the formation of gel is physiological stimuli, i.e., through pH, temperature, and ion-induced triggers.

### **Physiological stimuli**

#### **Temperature-triggered *in-situ* gel**

In some *in-situ* gel formulations, the sol-gel transition is triggered by a change in temperature. This mechanism relies on the thermal responsiveness of certain polymer or gelling agents. Liquid can be injected into the eye with precision and ease, without producing pain or blurriness [23]. After administration, the liquid is transformed into gel at a precorneal temperature (35°C) to withstand lachrymal fluid dilution without causing the drug to be rapidly eliminated from the precorneal [24]. The thermoreversible polymers undergo a transition from sol-gel above their lower critical Sol

temperature (LCST). LCST or lower consolute temperature is the critical temperature below which all the combinations are miscible. As the temperature of the Sol is raised above the LCST of the thermoreversible polymer, a critical temperature is reached [25]. At this point, the polymer undergoes a phase transition and becomes hydrophobic. This transition is driven by the dehydration of the polymer chains, causing them to collapse and aggregate. The collapsed polymer chains start to associate with each other forming a 3D network throughout the Sol [26]. This network entraps water and other solutes within its structure, resulting in the formation of the gel. The gelation process is highly reversible, it can be converted into a sol state when the temperature is lowered below LCST [27]. Poly-N-isopropylacrylamide (PNIPAAm) has benefits including high aqueous solubility and a LCST that is near to body temperature. PNIPAAm is not frequently employed, though, which may be due to potential drawbacks including low biodegradability and low drug loading capacity [28]. Elmotasem Heba *et al.* [29] formulated niosomal *in-situ* gel loaded with fluconazole to treat fungal keratitis using thermosensitive polymers such as poloxamer 407. The formulated gel showed enhanced corneal permeation with good entrapment efficiency (EE) and good gelling capacity with a gelation temperature of about 35.7°C [29]. Another interesting study performed by Gugleva *et al.* [30] formulated a niosome-loaded *in-situ* gel of doxycycline for ocular delivery using poloxamer 407 as a polymer alone and in combination with hydroxy propyl methyl cellulose (HPMC). The gelation temperature of the prepared gel was determined to be 34 °C, suitable for ocular delivery. *In vitro* investigation showed sustained release of drugs from the system [30].

#### **pH-triggered *in-situ* gel**

pH-sensitive *in-situ* gels are a type of advanced drug delivery system that undergoes gelation in response to changes in pH. These gels are particularly useful for controlled drug release in different parts of the body where pH levels vary. These gels contain biocompatible polymers that undergo pH-dependent conformational changes [31]. These polymers may contain acidic or basic groups that can ionize or deionize in response to variations in pH. In an acidic environment, polymers with the acidic group become protonated leading to increased repulsion between polymer chains, which promotes gelation. Conversely, in a basic environment, these polymers deprotonate, disrupting the cross-linking and causing the gel to revert to the sol state [32,33]. Significant potential is required for the *in-situ* pH-triggered gelling technology to maintain drug release and stability of pharmacological products. In this regard, Zafar *et al.* [34] formulated a pH-triggered niosomal loaded *in-situ* gel of moxifloxacin using chitosan to improve ocular residence time in the treatment of bacterial infection of the eye. Optimized formulation was evaluated for antimicrobial properties, gelling capacity, *Ex vivo* penetration, and *in vitro* drug release. Results showed good antimicrobial activity with a sustained release pattern and increase in permeability. It also showed good gelling capacity within seconds [34]. Allam and coworkers, [12] focused on developing a niosome-loaded *in-situ* gel of Betaxolol hydrochloride for the treatment of glaucoma to

increase precorneal residence of drug using Carbopol 934P and Hydroxy ethyl cellulose as a pH-sensitive polymer. The formed gel showed an *in-vitro* sustained release pattern. Whereas, the *in-vivo* study performed in rabbit eyes showed a remarkable decrease in intraocular pressure and significant advancement in bioavailability when compared to the marketed formulation [12].

#### **Ion-triggered *in-situ* gel**

An ion-triggered *in-situ* gel is a system that triggers gelation in response to changes in the ionic environment. Ion-induced gelation relies on the use of ion-responsive polymers, also known as ion-sensitive or ionotropic polymers [35]. These polymers possess functional groups or side chains that can interact with ions in the surrounding environment. These polymers often carry negatively charged groups, such as carboxylates (–COO<sup>-</sup>) or sulfate (–SO<sub>3</sub><sup>-</sup>) groups which can interact with positively charged ions (cations) in the Sol. This interaction involves electrostatic attraction between negatively charged polymer and positively charged ions [36]. The ion-polymer complex results in the formation of a 3D network, transforming a liquid Sol into a gel. The extent of gelation and gel properties can be controlled by adjusting the concentrations and type of ions added [37].

Banasubramaniam *et al.* [38] devised an ion-activated *in-situ* gelling system, for bacterial conjunctivitis to deliver ciprofloxacin hydrochloride for a longer duration of action. Gelrite, gellan gum was employed both alone and in combination with sodium alginate as the gelling agent. The formulations showed an 8-hours sustained drug release *in vitro* and were clinically effective [38]. Furthermore, Phenylephrine hydrochloride niosome-loaded *in-situ* gel was formulated using gellan gum as an ion-sensitive polymer. The formulation exhibited sustained drug release and was found to be effective in the treatment of mydriasis when performed *in vivo* study in the rabbit eye [39].

#### **Physical stimuli**

##### **Swelling triggered**

Swelling triggered *in situ* gel undergoes swelling forming a gel matrix that can sustainably release encapsulated drug over time due to changes in temperature or pH. In this approach, the material begins to gel as it takes water from the environment and then expands to fill the desired space. Polar lipid Myverol 18–99 (glycerol mono-oleate) expands in water to create lyotropic liquid crystalline phase formations. It has some bio-adhesive qualities and is susceptible to enzymatic degradation *in vivo* [40]. Myverol 18–99/water gel bio adhesion appeared to be caused by secondary chemical bonding, such as van der Waals forces but was restricted by their cohesive strength [41].

##### **Diffusion/Solvent exchange triggered**

This approach involves dispersing solvent from a polymer arrangement into adjacent tissue, which results in polymer grid precipitation. N-methyl pyrrolidone is used as a diffusion-triggered *in-situ* gel [42].

## Chemical Stimuli

### Ionic cross-linking

Polymers may undergo a phase transition in the presence of different ions. For example, gellan gum (an anionic polysaccharide) undergoes gelation due to the presence of various cations such as  $Mg^{+2}$ ,  $Ca^{+2}$ ,  $Na^+$ , and  $K^+$ , whereas pectins undergo gelation due to the presence of divalent cations. Likewise, Kappa-carrageenan forms a hard gel in response to a monovalent cation and forms an elastic gel in the presence of a divalent cation [43].

### Enzymatic cross-linking

To formulate a reversible gelling system, enzymatic cross-linking is the most effective technique. In this approach formation of gel occurs by cross-linking of enzymes present in body fluid. An enzymatic cycle operates effectively in physiologic conditions without the requirement for potentially hazardous chemicals such as monomers and initiators [44].

### Photoinitiated polymerization

To formulate *in-situ* gel, electromagnetic radiation can be used. With the use of electromagnetic radiation, the gel can be prepared by injecting a Sol of reactive macromere or monomers and invaders into a tissue location. Ideal polymers for polymerization are those that undergo dissociation of functional groups in the presence of light initiators like acrylate or similar monomers and macromers [45].

## POLYMERS USED IN THE FORMULATING REVERSIBLE GELLING SYSTEM

Ideal polymer is selected based on the type of *in-situ* gelling system that is to be formulated. Various types of

polymers can be used as per their characteristic as shown in Table 1.

### Nano formulations based on *in situ* gels

*In situ*, gel systems include a delivery vehicle made of polymers (natural, semi-synthetic, or synthetic) that have the unique feature of converting the sol into a gel when impacted by a biological stimulus [46]. Gels help the nanocarriers to be administered locally to the target tissue, and to maintain their release. When applied to the eyes, the formulation initially remains liquid, allowing for easy administration. However, upon contact with the ocular surface, it undergoes gelation, resulting in sustained drug release, improved drug absorption, and increased therapeutic effectiveness [47,48]. This approach is particularly useful in treating ocular conditions and diseases where prolonged drug releases and increased drug retention on the eye surface are essential for successful treatment [49]. Nano formulations such as liposomes, niosomes, and nanostructured lipid carriers are some novel methods of ocular delivery. Liposome as a carrier, is incorporated into an *in-situ* gelling system which undergoes phase transition upon contact with the ocular surface transforming into a gel-like consistency, increasing the retention time of the drug into the ocular surface which in turn increases drug bioavailability [50,51]. However, liposomes possess certain prevalent limitations such as a lack of targeting strategies, production challenges, stability, and poor drug loading capacity. Unlike liposomes, niosomes face issues of instability and aggregation, leading to changes in vesicle size and reduced drug encapsulation efficiency. By combining niosome with *in-situ* gel formulations, these challenges can be effectively addressed, leading to improved stability, drug

Table 1. Polymers of *In-situ* gel formulation.

Polymer	Characteristics	Ideal concentration	References
Poloxamer 407 (F-127)	Molecular weight-12000 Da Non-toxic Ethylene oxide (70% of the composition) contributes to its hydrophilic properties.	15%–20% w/v	[107]
Xyloglucan	Molecular weight-10000 Da. Obtained from the cell wall of a dicotyledonous plant. Highly water soluble.	1%–2% w/v	[108]
Carbopol 940	Molecular weight-20-600 kDa. Good mucoadhesive properties due to the presence of the carboxylic group. Exhibits a sol-to-gel transition at high pH levels.	0.5%–2% w/v	[109]
Gellan gum (Gelrite)	Ion-sensitive polymer Molecular weight-1,000,000 to 2,000,000. Undergoes a sol-to-gel transition with a divalent or monovalent cation like ( $Ca^{+2}$ , $Mg^{+2}$ , $K^+$ , $Na^+$ ). Thixotropic, Thermoplastic, and pseudoplastic behavior.	0.25%–0.75% w/v	[110]
Alginates	High guluronic acid content leading to good gelling capacity. Used for ion-a[117]ted system.	0.4%–1.5 % w/w	[111]
HPMC (Hypromellose, Methocel)	An increase in temperature causes an increase in viscosity.	1%–10% w/w	[112]

release, and overall efficacy in ocular drug delivery. The synergistic effect of both systems can enhance the therapeutic benefits of the delivered drug [52,53]. Nanostructure lipid carriers (NLCs) are colloidal drug delivery systems that consist of a blend of solid and liquid lipids. Formulating NLC-loaded *in-situ* gel for ocular delivery can address specific challenges associated with NLCs in the context of ocular drug delivery. Incorporating NLCs into an *in-situ* gel can provide a stabilizing matrix, reducing the likelihood of aggregation and enhancing the stability of the NLCs during storage and administration [54,55]. Table 2 gives details of some nano formulation-based *in-situ* gels for ocular application.

## NIOSOME LOADED IN-SITU GEL

Niosomes are the bilayer vesicles that can entrap amphiphilic drugs intended for ocular delivery within their aqueous core [56]. These nanosized vesicles are made up of a non-ionic surfactant [57]. These serve as carriers to protect the drug from degradation, improve solubility, and enhance its bioavailability. Gugleva and collaborators [58] formulated doxycycline hyclate niosome using different surfactants and cholesterol in different ratios using the thin film hydration method followed by a REV method. Their findings suggest that niosomes may be a viable drug delivery platform for doxycycline ophthalmic use [58]. Another interesting study was performed by Kattar *et al.* [59] on epalrestat niosomes to treat diabetic retinopathy by inhibiting the polyol pathway. Formulated niosomes were characterized for various parameters which proved niosomes to be a promising carrier to carry and encapsulate epalrestat through the eye in a controlled manner [59]. However, niosomes suffer from their physical fragility and the risk of drugs that may cause their poor precorneal retention [60]. This problem can be overcome by incorporating niosomes into the gelling system. Several investigations have revealed that this combination approach has considerably improved ocular bioavailability for niosomal-loaded *in-situ* gel [61]. Doxycycline-loaded niosomal *in situ* gel formulated by a modified thin film hydration method for treating corneal infections, keratoconjunctivitis sicca showed enhanced antibacterial activity and sustained *in vitro* drug release drugs, ensuring sufficient therapeutic concentration [30]. *In-vivo* studies of Vancomycin-loaded niosomal *in situ* gel performed in MRSA-infected rabbits showed an increase in antibacterial activity after treatment as compared with those rabbits that were treated with free drug Sol [62]. Niosomes-loaded *in-situ* gel offers a promising approach by addressing many of the challenges associated with conventional dosage forms and providing a more effective and patient-friendly option for treating various eye conditions and diseases [63]. Various research has been conducted based on *in situ* niosomes in ocular delivery as shown in Table 3.

### Factors affecting formulation of niosomes

#### Effect of type and structure of surfactant

The critical packing characteristics of surfactant can be used to predict the geometry of the vesicle to be generated. The type of micellar structure that forms can be determined from the critical packing parameter, if critical packaging parameter

(CPP)  $< \frac{1}{2}$  then spherical micelles are formed, if  $CPP > \frac{1}{2}$ , then bilayer micelles are formed, and if  $CPP > 1$ , then inverted micelles are formed. An increase in the alkyl chain length and transition temperature of the surfactant leads to an increase in the EE of the drug. An increase in concentration of surfactant beyond normal value leads to the formation of micelles [64]. The effect of surfactant in formulating niosomes is shown in Figure 1.

#### Effect of cholesterol

Cholesterol serves several important roles in the structure and properties of niosomes. Cholesterol helps in stabilizing the bilayer structure of niosomes. It is well-known for its capacity to intercalate between the alkyl chains of surfactant molecules, hence increasing the lipid bilayer's rigidity and stability. This can enhance the overall structural integrity of niosomes and prevent substances that are encapsulated from leaking [65]. The amount of cholesterol to be added is determined by the surfactant's hydrophilic-lipophilic balance (HLB) value. To account for the larger head groups, the minimum quantity of cholesterol that must be added must be increased as the HLB number rises over 10. The rate of encapsulated material release decreases when the stiffness of the bilayers increases in response to an increase in cholesterol content within the bilayers [66]. The kinetics of drug release may be affected by the cholesterol in niosomes. It may have a barrier effect, decreasing the permeability of the niosome membrane and therefore delaying the release of the medication that has been encapsulated. Cholesterol has two effects: it causes gel-state bilayers' chain order to decrease and liquid-state bilayers' chain order to increase [67].

#### Hydration medium

Additional crucial factors are the niosomes volume and the duration of hydration. The use of aqueous Sols with different pH levels and ionic strengths as hydration medium can affect vesicle properties. Improper hydration can result in the production of fragile niosomes or drug leakage from niosomes [68].

#### Temperature of hydration medium

The temperature of hydration affects the niosomes shape and dimensions. For best results, it should be higher than the gel-to-liquid phase transition temperature of the system. The temperature of the hydration medium is essential to optimize to obtain the proper niosome properties. Temperature variations have an impact on both the assembly of surfactants into vesicles and the modification of vesicle shape [69].

#### Nature of encapsulated drug

Molecular weight, lipophilicity, hydrophilicity, and chemical structure of drugs influence the size of niosomes. The hydrophilic drug decreases stability and increases leakage from vesicles, while the hydrophobic drug increases stability and decreases leakage from vesicles. Amphiphilic drugs decrease leakage from vesicles and increase encapsulation [70]. The entrapment of drugs in niosomes causes an increase in vesicle size, most likely as a result of the solute interacting with the

Table 2. Nano formulations based *in situ* gels for ocular delivery.

Formulations	Drug	Excipients	Method of preparation	Type of stimuli	Disease	Key findings	References
NP-based <i>in situ</i> ocular formulation	Oxytetracycline HCl	Poloxamer 407, Polyacrylic acid, gelatin, polyvinyl alcohol	Cold method	Thermosensitive	Bacterial keratitis	Increase concentration of poloxamer showed formation of stable gel and increases ocular contact time.	[113]
	Pioglitazone	Poloxamer 407, HPMC K4M, PLGA	Single emulsion solvent evaporation method	Thermosensitive	Dry eyes	Effective against <i>Pseudomonas aeruginosa</i> . Formulation was non-irritating and worked well to cure dry eyes.	[114]
	Ciprofloxacin	Eudragit RL 100, PVA, chitosan	Nanoprecipitation method	pH-dependent	Ocular infection	Extended corneal residence duration due to chitosan.	[115]
	Moxifloxacin	Gellan gum	Solvent evaporation	Ion sensitive	Ocular infection	Increasing contact time and showing stability. Reduced frequent administration.	[116]
Liposomes based <i>in situ</i> formulations	Methazolamide	Carbopol 934	Lipid film hydration	Ion triggered	glaucoma	Significant decrease in intraocular pressure.	[117]
	Travoprost	Gellan gum, carbopol 934	Conventional thin film hydration method	Ion sensitive	glaucoma and ocular hypertension	Travoprost liposomal <i>in situ</i> gel showed less irritation, increased bioavailability.	[118]
	Timolol maleate	Gellan gum	REV	Ion sensitive	glaucoma	Effective in reducing intraocular pressure when compared to the marketed formulation.	[119]
	Fasudil hydrochloride	Poloxamer 407, HPMC K4M	Solvent evaporation technique	Thermosensitive	glaucoma	Enhanced corneal permeability with better tolerability for management of glaucoma.	[120]
Nanoemulsion based <i>in situ</i> formulations	Acetazolamide	Gellan gum, xanthan gum, Carbopol, HPMC	High energy method	Ion triggered	Glaucoma	Gellan/xanthan and gellan/HPMC showed greater therapeutic efficacy and a longer-lasting effect in lowering intraocular pressure.	[121]
	Acyclovir (ACV)	Poloxamer 407, poloxamer 188	Low energy method	Thermosensitive	Herpetic simplex keratitis	Showed high corneal drug penetration and facilitating adequate uptake of ACV.	[122]
	Brinzolamide	Gellan gum	High energy method	Ion triggered	Glaucoma	Well tolerated and resulted in dec [116] in intraocular pressure.	[123]
NLC based <i>in situ</i> formulations	Curcumin	Chitosan	Hot melt emulsification, ultrasonication techniques	pH-dependent	Ocular inflammation	Showed enhanced corneal penetration and 2.5 times greater flux than the control suspension of curcumin propylene glycol.	[124]
	Ciprofloxacin and natamycin	Poloxamer 188, gellan gum	Homogenization method	Temperature sensitive	Microbial keratitis	Dual nanoparticle-based <i>in-situ</i> gelling medication delivery method for treating eye infections.	[125]
	Bimatoprost	Acrypol 941	High shear homogenization	pH-sensitive	glaucoma	Non-irritant and showed the drug release for a prolonged period without damaging the cornea.	[126]
	Triamcinolone acetonide	Pluronic F68, gellan gum	Hot homogenization, ultrasonication	Temperature-dependent	Inflammation, posterior uveitis, diabetic macular edema.	Reduced pre-corneal drug loss, demonstrating enhanced permeability and extended residence duration in the conjunctival sac.	[127]

Table 3. Niosomal *in situ* gels for ocular administration.

Drug	Surfactant	Method of preparation	Stimulus	Key findings	Reference
Doxycycline	Span 60, Poloxamer 407	Modified thin film hydration method and cold method	Temperature sensitive	Gelation temperature of 34°C, suitable for ophthalmic preparation.	[30]
Prednisolone sodium phosphate	Span 60, Poloxamer 407, and poloxamer 188	Thin film hydration method and cold method	Temperature sensitive	Good stability and pseudoplastic flow behavior, with sustained therapeutic concentration and drug release. Enhanced ocular bioavailability. <i>In vivo</i> experiments in rabbits revealed a greater concentration of medication in aqueous humor with no sign of discomfort.	[128]
Natamycin	Span 60, Poloxamer 407	Thin film hydration method and cold method	pH sensitive	The bio-adhesive characteristic increased corneal retention time and displayed longer drug release of up to 24 hours when compared to the marketed formulation.	[76]
Timolol maleate	Chitosan and Carbopol	REV and cold method	pH-sensitive	sustained release for up to 8 hours and a decrease in IOP when compared to the marketed formulation.	[129]
Dexamethasone sodium phosphate	HPMC (K4M), Carbomer 974 P	The solvent injection method and cold method	pH-sensitive	entrapment efficiency of 93.15% with no sign of irritation, longer residence duration, and ability to sustain the drug release.	[78]
Vancomycin	HPMC, poloxamer 188, poloxamer 407	Lipid film hydration and cold method	Thermosensitive	<i>In vivo</i> , study performed in MRSA-infected rabbits showed an increase in antibacterial activity after treatment with vancomycin niosomal gel compared to those treated with the free drug solution.	[62]
Fluconazole	HPMC, Poloxamer 407	Thin film hydration and nanoprecipitation techniques	Thermosensitive	Good entrapment efficiency with enhanced corneal permeation and sustained drug release, proving them a novel approach for treating eye-threatening fungal infections.	[29]
Latanoprost	Pluronic F127, HPMC	REV and cold method	Thermosensitive	<i>In vivo</i> experimental results showed a decrease in the intraocular pressure in rabbit eyes with no sign of irritancy and increasing bioavailability in comparison to the commercially available latanoprost eye drops	[3]
Pilocarpine hydrochloride	Locust bean gum, Carbopol 934	Ether injection method and cold method	pH-sensitive	Extended preclinical residence duration and improved bioavailability.	[130]
Ketotifen	Gellan deacetylase gum	REV and cold method	Ion sensitive	Deacetylase gellan gum extended the formulation's residence period, according to scientific investigations.	[131]
Betaxolol	HEC, Carbopol 934 P	Thin film hydration method and cold method	pH-sensitive	high entrapment effectiveness and a long-lasting <i>in vitro</i> drug release pattern when injected into the eye of a rabbit, leading to reduced intraocular pressure with enhanced drug bioavailability.	[12]
Brimonidine	HPMC K15M Carbopol 940	Thin film hydration and cold method	pH-sensitive	good antiglaucoma activity with sustained effect in reducing intraocular pressure than marketed and niosomal drops.	[132]
Curcumin and gentamicin sulfate	Poloxamer, chitosan	Thin film hydration and cold method	Thermosensitive	High entrapment efficiency and appropriate physicochemical parameters with suitable rheological and gelling characteristics.	[133]
Moxifloxacin hydrochloride	Chitosan	Thin film hydration and cold method	pH-sensitive	Good antimicrobial properties with a sustained release profile and good bio-adhesion in tear film without any toxicity to the tissue in comparison to pure moxifloxacin.	[34]
Flurbiprofen	Poloxamer 407, HPMC K4M	Thin hydration method and cold method	Thermosensitive	High <i>in situ</i> gelling capacity with long-t [129]rug release over 8 hours.	[134]
Phenylephrine hydrochloride	Gellan gun and HEC	Cold method	Ion-sensitive	sustained drug release and was found to be effective in the treatment of mydriasis when performed <i>in vivo</i> study in the rabbit eye.	[39]
Brinzolamide	Poloxamer P 407, kolliphor P 188	Thin film hydration method	Thermosensitive	significant decrease in intraocular pressure by releasing drug in a sustained manner.	[135]

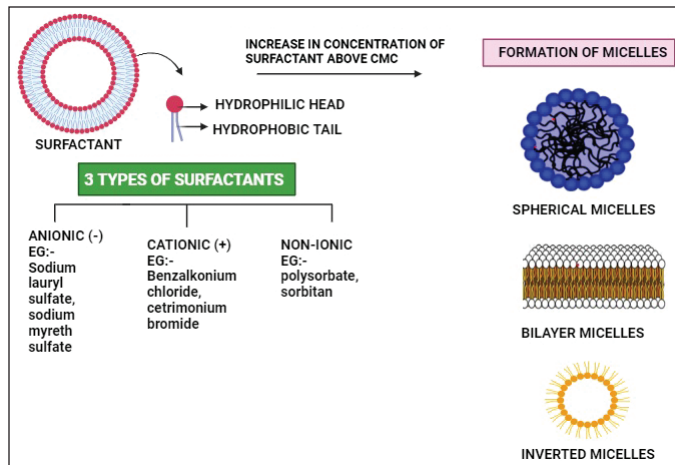


Figure 1. Effect of surfactant in formulating niosomes.

head groups of the surfactant, which raises the charge and mutual repulsion of the surfactant bilayers [68].

#### Drug loading method

The efficiency of drug loading can be affected by the process utilized to incorporate medications or active ingredients into niosomes [71]. Based on the properties of the encapsulated substance, techniques such as remote loading or post-insertion can be used [71]. A study [72] has been conducted on niosome preparation techniques, including sonication, ether injection, and handshaking. Vesicles formed by the handshaking approach had a larger diameter (0.35–23 nm) than those formed by the ether injection method (50–1000 nm). The REV method can produce small-sized niosomes. The microfluidization process results in more homogeneity and smaller vesicles [72].

#### METHOD OF PREPARATION OF NIOSOMAL LOADED *IN SITU* GELS

Niosome-loaded *in situ* gel involves a combination of two distinct drug delivery approaches together. Incorporation of drug-loaded niosome in the *in-situ* gel offers advantages of both dosage forms. In the first step of niosome-loaded *in situ* gel formation, niosomes are prepared by any method such as the handshaking method, REV, or other injection method as shown in Figure 2. The second step involves formulation of *in situ* gel by accurately weighing and mixing *in situ* polymer with a suitable amount of water or buffer Sol to form a gel and adjust pH if needed. In the final step, the drug-loaded niosomes are incorporated into the *in-situ* gel formulation with continuous stirring as shown in Figure 2. This uniform dispersion was kept overnight at 4°C to get a clear Sol. Formulated niosomes loaded *in situ* gel are then characterized for various evaluation parameters [73–76].

#### CHARACTERIZATION

Characterization of niosome-loaded *in situ* gel is a comprehensive process that involves accessing physicochemical properties, drug release kinetics, safety, and efficacy. A thorough evaluation is crucial for optimizing the formulation and ensuring its suitability for delivering drugs with enhanced

therapeutic outcomes. In addition, quality control standards and regulatory guidelines should be followed to guarantee the safety and efficacy of this drug delivery system.

#### In-vitro evaluation

##### pH

Measuring the pH of niosome-loaded *in-situ* gel for ocular delivery is an important parameter for ensuring the stability and compatibility of the formulation with the ocular environment. Immerse the pH meter in a sample and allow it to stabilize to display a stable pH reading. Be sure to take multiple measurements and calculate the average for accuracy. Ideally, ophthalmic formulations must possess a pH equivalent to lacrimal fluid value, i.e., 7.4 [77].

##### Gelling Time

The time taken by the formulation to convert into gel is noted. Gelling time should be in the range of 5–7 seconds. The gelling time of the sample will be tested according to the method described by Patel *et al.* [78] using the test tube inversion method. This method involves transferring of sample into the test tube and placing it in a water bath at 35°C ± 0.5°C. The test tube was inverted at 90°C and the time when no fluidity of the sample was observed was evaluated as the gelling time.

##### Gelling capacity

A drop of prepared niosome-loaded *in-situ* gel is placed in a vial containing freshly developed simulated tear fluid (STF) (sodium chloride, 0.67 g, sodium bicarbonate, 0.20 g, and calcium chloride dihydrate, 0.008 g in 100 ml of distilled water) to determine the prepared formulation's gelling capability. The gelling capability of the Sol was assessed based on the stiffness of the created gel and the time duration during which the formed gel remains thus [78].

##### Isotonicity adjuster

For ocular delivery, isotonicity is a crucial factor to ensure that the formulation does not damage or irritate ocular tissues. The formulation was mixed with a few drops of blood to determine isotonicity, and the morphology of the blood cell was studied under a microscope. Blood cells retain their integrity in isotonic fluids, whereas cells shrink in hypertonic and bulge in hypotonic Sols [79].

##### Viscosity

Measurement of viscosity is critical for assuring the consistency and quality of *in-situ* gels in manufacturing operations. Consistent viscosity aids in the production of gels with the desired qualities and performance characteristics. Viscosity can be measured by using a Brookfield viscometer. After allowing the prepared system to gel in the STF, the viscosity is measured at various angular velocities [80].

##### Polydispersity index (PDI), and zeta potential (ZP)

Dynamic light scattering and Malvern zeta sizer are used to analyze the PDI and ZP of niosome-loaded *in-situ* gel. The homogeneity of the size distribution was measured



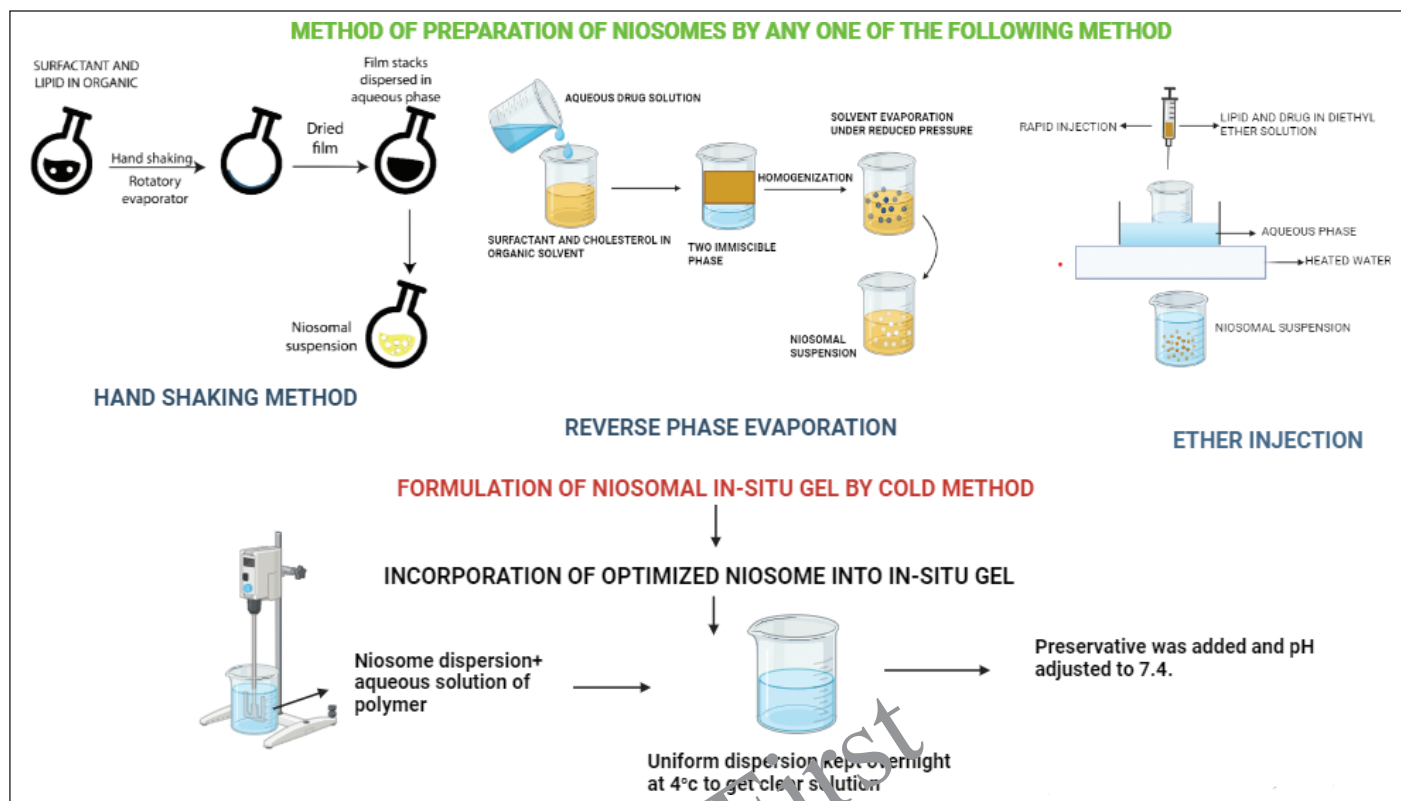


Figure 2. Different method of preparation of niosomes loaded *in situ* gels.

using PDI. A lower PDI value indicates a narrower size range, implying more uniform particle sizes (PSs). The PDI scale goes from 0 (completely monodisperse) to 1 (extremely polydisperse). A higher ZP value indicates better stability because it suggests strong repulsive forces between particles, preventing aggregation. A ZP value of  $\pm 30$  mV or higher is often considered desirable for good stability [80].

### EE

It is defined as the total amount of drug entrapped inside the vesicle. It can be determined using the centrifugation method. Centrifuge the niosomal *in-situ* gel at a specific speed and duration that separates the niosomes from the gel. After centrifugation two fractions are obtained: one is the precipitate containing niosomes and the other is the supernatant containing an unencapsulated drug. Carefully collect the supernatant and measure the amount of drug in the collected supernatant by diluting the supernatant and accessing it in HPLC or UV-VIS spectroscopy. Calculate EE using the following formula:

$$\% EE = \frac{\text{Total amount of drug-untrapped drug}}{\text{total amount of drug taken}} * 100.$$

Higher EE indicates that a larger portion of the drug is encapsulated within the niosomal *in-situ* gel, which is typically desirable [81].

### *In-vitro* drug release study

*In vitro* drug release study is carried out using a Franz diffusion cell or by using dialysis method.

**Dialysis method** This method typically involves encapsulating the drug formulation within a semipermeable membrane, followed by immersion in a release medium or physiological buffer Sol. The semi-permeable membrane acts as a barrier that permits the passage of the drug molecule while retaining the drug carrier or any other large molecule present in the formulation. This membrane is then placed in the dialysis bag and the release medium is constantly stirred to ensure uniform condition throughout the experiment. Release of the drug from the formulation is monitored at specific time intervals by analyzing the drug concentration in the release medium using UV-visible spectroscopy, HPLC, and so on [82,83].

**Franz diffusion cell** It is the most widely accepted apparatus for conducting *in-vitro* drug release studies. This method allows for the evaluation of the rate and extent of drug release from formulation through synthetic membranes, often mimicking the biological barrier. The apparatus consists of two compartments, i.e., the donor compartment that contains drug formulation, while the receptor compartment contains a suitable medium. The procedure involves placing drug formulation on the surface of the membrane in the donor compartment and maintaining the receptor compartment under constant stirring to ensure uniform drug concentration. Samples are collected at a predetermined interval from the receptor compartment for analysis using techniques such as UV-visible spectroscopy, HPLC, or other suitable methods depending on the nature of the drug [84,85].

### Morphology study

Microscopical methods, including transmission electron microscopy (TEM) and scanning electron microscopy (SEM), can be used to analyze the morphology of niosomal vesicles. For SEM characterization, the niosome-loaded *in-situ* gel should first be transformed into a dry powder, which is put on a sample holder and then coated with a conductive metal (sputter coating either with gold, or gold-palladium). The coating prevents charging effects during the SEM analysis. Set the appropriate voltage and beam current suitable for the gel under investigation. For TEM analysis samples were diluted using distilled water. A droplet of the specimen was positioned on a copper grid coated with carbon, dyed with 2% phosphotungstic acid, and allowed to dehydrate. The photomicrographs demonstrate that the niosome-loaded *in situ* gel was distinct, spherical, and free of aggregations with a smooth surface [29].

### Ex-vivo evaluation

#### Ex-vivo corneal permeability

Freshly removed rabbit corneas were used in a Franz diffusion cell for *ex-vivo* corneal permeability research. The dissected rabbit cornea was placed facing the donor compartment [29]. It was then placed on a magnetic stirrer set to 100 rpm and maintained at a constant temperature of 35.1°C. The chosen formulations were applied to the ocular surface of the donor compartment, and the receptor medium was isotonic phosphate buffer (pH 7.4). At prearranged intervals, aliquots of the receptor medium were taken out of the sampling port and replaced with an equivalent volume of fresh medium to maintain a consistent volume. Spectrophotometric analysis was performed on the samples [29]. The permeability coefficient ( $P$ ) was calculated using the following formula:

$$P = (dQ/dt)/AC = J/C$$

$$dQ/dt = \text{Rate of permeation}$$

$$A = \text{Surface area of diffusion membrane}$$

$$J = \text{Slope}$$

$$C = \text{concentration of drug in the donor compartment.}$$

### In-vivo evaluation

#### Eye irritancy test (Draize test)

The Draize test is a typical method for determining the potential irritancy and toxicity of chemicals, especially those that may encounter the eyes. While performing the Draize test in rabbits it is important to note ethical and animal welfare considerations [86]. Healthy adult rabbits free from ocular injuries are selected for study. A small amount of the test chemical is directly injected into the rabbit's conjunctival sac. Over many days, the rabbits are observed at predetermined intervals, noting any indications of discomfort, inflammation, or unpleasant reactions. The rabbits should receive the proper post-test care, including any necessary medication for any negative test results [87].

### Histopathology

A histopathology study is performed to verify the safety profile of the formulation. The sample of tissue or cells that have been exposed to the formulation is collected and placed in the formalin Sol for 24 hours to maintain structural integrity. Tissue is then bisected and mounted in a paraffin block; this process is known as paraffin embedding. The section is transferred to a microscope slide and stained with hematoxylin and eosin to visualize tissue morphology and cellular structure. The histopathological alterations brought on by the *in-situ* gel filled with niosomes are analyzed [88].

### Stability studies

Niosomes loaded *in-situ* gel must undergo a stability study to assess the formulation's long-term physical, chemical, and biological stability. Storage of the niosome-loaded *in-situ* gel is observed over three months at two distinct temperatures (4°C in a refrigerator and 25°C). Samples are collected every two, and three months, and any physical alterations in the PS, PDI, ZP, color, smell, or percentage of EE were assessed. Three measurements of each parameter were made, and the mean value will be calculated [63,89]. A stability study performed by A.A. Omnia *et al.* [90] on levofloxacin-loaded niosomal *in-situ* gel when kept in various storage situations, revealed no changes in appearance. There was a negligible change (0.05) in the EE%, PS, PDI, or ZP at 4°C indicating good stability compared to freshly prepared formulation [90].

### TOXICITY OF NIOSOMES

Surfactants are widely used in the formulation of niosomes. These amphiphilic molecules are used to construct the bilayer membrane of the niosomes. Based on charge, surfactants can be classified into ionic and non-ionic surfactants. Ionic surfactants are of three types- anionic, cationic, and amphoteric. Ionic surfactants have limited ocular applications because of toxicity issues. Ocular tissues are extremely sensitive, and their intricate structure necessitates great caution when choosing components for ocular administration. Surfactants potentiate to cause corneal and conjunctival cellular changes, especially at high concentrations [91]. Cationic surfactants like benzalkonium chloride are responsible for corneal epithelial cell death. A study assessing the cytotoxicity of different surfactants on rabbit corneal cells found nonionic surfactants to be the least irritant, followed by amphoteric, anionic, and finally cationic surfactants to be the most irritant [92]. Surfactants with polar head groups that are not electrically charged are known as non-ionic surfactants.

Brij, polyglycerol alkyl ethers, ester-linked surfactants, glucosyl dialkyl ethers, Spans (sorbitan esters), crown ethers, Tweens (polysorbates), and polyoxyethylene alkyl ethers are examples of non-ionic surfactants used to manufacture niosomes. These surfactants are classified as generally recognized as safe by United State Food and Drug Administration. As previously stated, nonionic surfactant contains no charge hence, are considered to be less irritating and less toxic compared to other surfactants but, can still potentially cause mild irritation when coming into contact with sensitive

areas such as the eyes. The specific toxicity of non-ionic surfactants to the eyes depends on various factors, including the concentration of surfactants, the duration of exposure, chemical structure, and individual sensitivity [93]. Non-ionic surfactants are toxic for  $LC_{50}$  between 1 and 10 mg/l [94]. In a study, performed by Hamad Alyami and co-workers formulated non-ionic surfactant vesicles of pilocarpine hydrochloride for ocular drug delivery and the purpose of the study was to characterize the toxicological profile of non-ionic surfactant (span 60). The toxicity of optimized niosomes was tested on the human corneal epithelium-2 cell line as a model of corneal epithelium. Results showed no corneal cellular toxicity with a span 60 [52]. Another interesting study conducted in 2012 by Abdelkader assessed the ocular toxicity of niosomes by examining the potential for conjunctival and corneal irritation caused by Span 60 niosomes and Span 60 niosomes containing ranging proportions of bilayer membrane components such as Solulan C24, dicetyl phosphate, and sodium cholate. They did this by using an acceptable *in vitro* conjunctival model (hen's egg chorioallantoic membranes) and excised bovine corneal opacity and permeability models. The results of this investigation indicate that niosomes can be tolerated by the eyes with minimum ocular irritations [95].

## CORNEAL PENETRATION AND ABSORPTION MODELS

In the prior stages of formulation development, robust *in vitro* and *Ex vivo* models are needed to generate more effective drug carriers. Compared to *in vivo* experiments, these techniques are more expedient, less expensive and more humane. The standard protocol for conducting corneal permeation investigations involves preparing the tissue either *in vitro* or *Ex vivo* and then putting it in an assembly, like a Ussing chamber or a Franz diffusion cell [96-97]. Transcorneal penetration investigations are increasingly being conducted using *in vitro* cell culture models. Studies on corneal penetration

can be greatly aided by artificially created human corneal equivalents, even if research and development on these corneas are still underway [98]. *In vitro* and *Ex vivo* models frequently aid in reducing the number of laboratory animals utilized and have proven to be a more pertinent, affordable, efficient, and ethical option for investigating ocular formulation absorption and penetration [53]. Various corneal permeation and absorption models utilized are as follows:

- 1) *In-vitro* models
- 2) *Ex-vivo* models.

### IN-VITRO MODELS

The *In-vitro* model serves to reduce the associated cost and number of laboratory animals employed for permeation studies. Rabbit, rat, bovine, human, and porcine corneal cultures are generally utilized for this purpose. Rabbit cells are readily used due to their easy availability whereas, human cells are difficult to obtain. To date, a variety of *in vitro* models have been developed, each model has its pros and cons. *In vitro*, models as specified in Table 4 can be broadly categorized into three groups: immortalized cell line, reconstructed tissue culture, and primary cell culture [99]. Primary cultures do not fully represent ocular tissue since they are often multilayered (5–6 layers) cell cultures of a single kind of cell. The cells in immortalized cell lines have undergone genetic modification [66]. Usually, tumors are the source of these cell lines, or they are created through viral oncogene transformation. Reconstructed tissue culture entails creating an analogous human cornea composed of endothelial, stromal, and epithelial cells [100]. Isolated ocular tissues and corneal epithelial cell cultures from rabbits and pigs have been used by Scholz *et al.* [101] to examine the penetration of the hydrophilic drug pilocarpine hydrochloride (p-HCl). A strong correlation was observed in P-HCl transport across the isolated tissues and cell cultures [101]. Human corneal epithelial cells (HCE-T), Statens Serum Institut rabbit

Table 4. *In-vitro* corneal permeation and absorption model.

<i>In-vitro</i> model	Cells/tissue	Advantages	Disadvantages	Reference
<b>Primary culture</b>	Rabbit/rat corneal cell	Inexpensive and readily accessible	The result may vary for different compounds and epithelial cells used are not reliable representatives of the entire cornea.	[101]
<b>Reconstructed tissue culture</b>	Bovine corneal tissue	The morphology of excised and reconstructed corneas is comparable, and drug permeation occurs in the same order for both.	Penetration of drugs across the cornea is much faster as compared to the excised cornea.	[136]
	Human and porcine corneal tissue	Porcine cornea is functionally and morphologically equivalent to the human cornea and is found to be an efficient model in determining irritation and toxicity.	Permeation of drugs through the human cornea is more as compared to the porcine cornea due to the thinner epithelium of the human cornea.	[137]
<b>Immortalized cell lines</b>	Araki-sasaki cell line	It mimics human corneal epithelium and is an efficient model for studying ophthalmic penetration and toxicity.	They proliferate endlessly and may exhibit distinct gene patterns that are distinct from all <i>in vivo</i> cell types.	
	Transformed human corneal epithelial cells (HCE-T)	Good correlation with excised rabbit corneas.	Method is not versatile and cannot be used for all drugs.	[138]
	Clonetics human corneal epithelium (C-HCE)	Model drug permeability through C-HCE correlates with dissected rabbit cornea and can be used to examine drug permeation.	C-HCE is anticipated to have reduced active efflux and uptake transport.	[102]

**Table 5.** *Ex-vivo* corneal permeation and absorption model.

<i>Ex-vivo</i> tissue	Advantage	Disadvantage	Reference
Rabbit	Reliable model for conducting permeation and absorption studies due to anatomical similarities with human eye and higher permeation as rabbit cornea is thinner as compared to the human cornea and lacks Bowman's layer.	<i>Ex-vivo-in vivo</i> correlation must be established.	[139,140]
Porcine	It is easily accessible, large, and durable.	Since the cornea of a pig is substantially thicker than that of a human, porcine corneal penetration is thought to be lower than that of a human cornea.	[141,142]
Bovine	An effective model for simulating ocular absorption investigations.	Poor permeability due to thicker epithelium of bovine cornea when compared to human cornea.	[143]

**Table 6.** Patent on *in-situ* gel for ocular delivery.

Patent number	Patent title	Year	Key findings	Reference
US2002/0114778	Ocular drug delivery using reversible gelling system	2002	Composition of an <i>in-situ</i> gel that effectively prolongs the residence period of the medicament in the eye while being more pleasant and simpler to administer.	[144]
US2003/6511660B1	Ophthalmic drug delivery formulations and techniques of preparing them	2003	This formulation exhibits low viscosity and free flow in non-physiological environments. It transforms into a strong gel at physiological conditions by an <i>in-situ</i> phase transition.	[145]
US2004/6703039B2	Ocular drug delivery using a reversible gelling system	2004	The formulation extends medication residence duration on the ocular surface by releasing the drug in a sustained way.	[146]
US2011/0082128 A1	Ophthalmic drug delivery system of estradiol or other estrogen for prevention of cataracts	2011	Formulation of estrogen-based <i>in situ</i> gel for the treatment of cataracts. In-vivo studies showed the delayed release of the drug to the eye, especially to the lens.	[147]
WO2011/018800A3	<i>In situ</i> gel-forming solution for ocular drug delivery	2011	Formulation of an <i>in-situ</i> gel by using a thermoreversible polymer and a combination of natural polysaccharides to increase bioavailability.	[148]
US2013/8343471B2	Gellan, PVA, and TPGS nanoparticulate <i>in-situ</i> gels as alternatives to vitreous humour	2013	This technology relates to the nanoparticulate vitreous substitute that is capable of delivering ocular drugs and producing good therapeutic responses.	[149]

**Table 7.** Marketed formulation of reversible gelling system for ocular delivery.

Name of product	Drug	Type of <i>in-situ</i> system	Polymer	Approval by FDA	Targeting disease	Manufacturer	Reference
Pilopine HS ophthalmic gel-4%	Pilocarpine hydrochloride	pH triggered <i>in-situ</i> gel	Carbopol 940	1984	Control intraocular pressure	Alcon Laboratories. Inc., USA	[35]
Timoptic-XE ophthalmic gel forming solution-0.25%, and 0.5%		Ion-induced	Gellan gum	2003	Ocular hypertension	Merck & Co. Inc.	[150]
Timoptol-LA Ophthalmic gel-forming solution-0.25%		Ion activated	Gellan gum	2004	Glaucoma	Merck sharp & Dohme Ltd.	[35]
Azasite ophthalmic solution-1%	Azithromycin	Temperature triggered	Poloxamer 407	2007	Bacterial infection	InSite Vision, Inc.	[150]
Akten ophthalmic gel-3.5%	Lidocaine hydrochloride	Temperature triggered	HPMC	2008	Local anaesthetic	Akorn Inc., Lake Forest, IL	[35]
Viragn ophthalmic gel-0.15%	Ganciclovir	pH triggered	Carbopol 974	2009	Fungal keratitis	Laboratories THEA-France	[151]

corneal (SIRC) cells, SkinEthic human corneal epithelium (S-HCE), and Clonetics human corneal epithelium (C-HCE) were compared with excised rabbit and human corneas in terms of their permeability and barrier characteristics by Becker *et al.* [102]. According to their findings, SIRC and S-HCE's barrier characteristics were not sufficiently developed to distinguish

between the permeability of distinct drug compounds because of leaky gap junctions [102].

### EX-VIVO MODELS

*Ex vivo* ocular models are a significant tool for studying corneal penetration and developing ocular medication delivery

techniques. Since human corneas are usually exclusively used for transplantation, *ex-vivo* models usually use removed animal corneas in the proper diffusion cell. In *ex-vivo* models, rabbit, porcine, and cow corneas are the most often employed tissues [103]. Table 5 discusses the most often utilized tissues in *ex-vivo* models. Despite being smaller than human eyes, rabbit eyes are the most popular choice for *Ex vivo* models. However, rabbits have substantially greater penetration because their eyes lack Bowman's layer. Pig's eyes and human eyes are most comparable structurally. Contrarily, the size of a cow's eye and its corneal epithelium are nearly twice as huge as those of a human being [103]. Ghada A. Abdelbary *et al.* [104] performed an *ex-vivo* corneal permeation study of proniosomal gel for ocular delivery on excised rabbit's cornea using a Franz diffusion cell. A significantly higher permeability coefficient ( $p < 0.05$ ) and a steady state flow of 2.44 mcg/cm<sup>2</sup>h and 0.000244 cm<sup>2</sup>/h, have been observed respectively [104]. Ex vivo experiments on fresh and thawed corneal tissues of pigs by Aller Rodriguez *et al.* [105] discovered that transcorneal penetration of cyclosporine A prodrug is a simple and reliable model.

### Future prospective

A combination of niosome and *in-situ* gels has emerged as a compelling area of research in the realm of pharmaceutical sciences. With their unique properties, this amalgamation presents a plethora of opportunities for advancing drug delivery mechanisms [61,35]. The future of niosome-loaded *in-situ* gel holds significant promise in revolutionizing therapeutic approaches, ensuring targeted and controlled drug delivery, and enhancing patient compliance [11]. With the continuous progress in nanotechnology and pharmaceutical sciences, the future of niosome-loaded *in-situ* gel envisages the development of personalized medicine. Beyond their pharmaceutical applications, niosome-loaded *in-situ* gels hold promise in the fields of biomedical research and cosmetics [106,26]. Some of the patented and marketed formulations of reversible gel are shown in Tables 6 and 7.

### CONCLUSION

The incorporation of niosome into an *in-situ* gel system provides a novel and synergistic method for boosting the distribution of therapeutic agents, overcoming the drawbacks of traditional drug delivery methods, and enhancing patient outcomes. Niosomes' capacity to encapsulate a variety of pharmaceuticals and the *in-situ* gels' ability to gel at different temperatures or pH levels enable fine control over drug release kinetics and the localization of drug activity. Niosome-loaded *in situ* gel has proven tremendous potential in several biological applications due to its combined benefits, which include improved stability, targeted drug delivery, sustained release, and increased bioavailability. Furthermore, niosome-loaded *in-situ* gel systems have shown promise in overcoming biological barriers, facilitating localized and sustained drug release at the target region, and encouraging prolonged residence time, notably in mucosal and ocular drug delivery applications. At present there is no marketed formulation based on niosome *in situ* gels for ocular delivery. Therefore, to make optimal use of this novel strategy and to satisfy the changing healthcare needs of various patient populations, multidisciplinary research and collaboration

efforts are necessary. There is an urgent need to explore the potential of this delivery method using clinical trials.

### List of Abbreviations

CPP: Critical packaging parameter; EE: Entrapment efficiency; HLB: Hydrophilic-lipophilic balance; HPLC: High-performance liquid chromatography; HPMC: Hydroxy propyl methyl cellulose; LCST: Lower critical solution temperature; NLC: Nanostructure lipid carrier; PDI: Polydispersity index; PNIPAAm: Poly-N-Isopropylacrylamide; PS: Particle size; REV: Reverse phase evaporation; SEM: Scanning electron microscopy; Sol: Solution; STF: Simulated tear fluid; TEM: Transmission electron microscopy; ZP: Zeta potential.

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### AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

### DATA AVAILABILITY

All data generated and analyzed are included in this research article.

### USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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