




REVIEW

Natamycin Ocular Delivery: Challenges and Advancements in Ocular Therapeutics

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ABSTRACT

Fungal keratitis, an ocular fungal infection, is one of the leading causes of monocular blindness. Natamycin has long been considered the mainstay drug used for treating fungal keratitis and is the only US Food and Drug Administration (USFDA)-approved drug, commercially available as a topical 5% w/v suspension. Furthermore, ocular fungal infection treatment takes a few weeks to months to recover, and the available marketed antifungal suspensions are associated with poor residence time, limited bioavailability (< 5%) and high dosing frequency as well as minor irritation and discomfort. Despite these challenges, natamycin is still the preferred drug choice for treating fungal keratitis, as it has fewer side effects and less ocular toxicity and is more effective against *Fusarium* species than other antifungal agents. Several novel therapeutic approaches for the

topical delivery of natamycin have been reported to overcome the challenges posed by the conventional dosage forms and to improve ocular bioavailability for the efficient management of fungal keratitis. Current progress in the delivery systems uses approaches aimed at improving the corneal residence time, bioavailability and antifungal potency, thereby reducing the dose and dosing frequency of natamycin. In this review, we discuss the various strategies explored to overcome the challenges present in ocular drug delivery of natamycin and improve its bioavailability for ocular therapeutics.

Keywords: Fungal keratitis; Marketed formulations; Natamycin; Novel formulations; Ocular delivery; Patents

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Key Summary Points

Fungal keratitis is a one of the leading causes of monocular blindness and is associated with the poor management of the disease.

This article offers the compressive review of modern approaches for the topical delivery of natamycin for efficient management of fungal keratitis.

Nanotechnology-based modern approaches offer improved bioavailability and efficacy at reduced dose and dosing frequency.

Much research has been done on natamycin delivery; however, no alternative dosage forms have reached the clinical stage, and the safety aspects need to be addressed.

INTRODUCTION

Fungal or mycotic keratitis (FK) is a severe fungal ocular infection that infects the clear transparent corneal surface as well as the immediately associated layers (stroma, Descemet's membrane and finally the anterior chamber). Acute fungal keratitis usually causes blurred vision owing to infection and corneal inflammation. The acuity can improve with treatment, but corneal scarring generally leaves the patient with worse acuity than before [1]. Recent investigations have reported that there are nearly 1.5 million new cases of fungal keratitis every year, and it is one of the corneal diseases that are leading causes of monocular blindness [2]. Fungal keratitis cases are most prevalent in the subtropical and tropical regions (20–60%) and developing countries, which may be largely related to the climate (e.g., high temperatures and relative humidity) and to any ocular trauma related to agriculture-related activities [3]. Several cases of fungal keratitis

have been associated with ocular steroid [topical corticosteroids (dexamethasone)] treatment, recent surgery, ocular trauma, occupational ocular injury, using contact lenses and diseases like diabetes mellitus and HIV [4–6]. The causative organisms are species (sp) of fungi such as yeasts (*Candida*), filamentous fungi with septae (*Fusarium*, *Aspergillus*, *Curvularia*, *Cladosporium*) and filamentous fungi without septae (*Rhizopus*) [6]. About 24–30% and 37–62% cases of FK are caused by *Aspergillus* and *Fusarium* sp., respectively. Due to the fast-advancing nature of *Fusarium*-related keratitis, the cases are more severe and less responsive to drug therapy than those of *Aspergillus* keratitis [6, 7].

Currently, three classes of antifungal agents are used to treat FK: triazoles, polyenes and echinocandins [8]. Natamycin (Nat) is a natural tetraene antibiotic also known as pimaricin, which belongs to a large class of polyene antifungals obtained by the fermentation of *Streptomyces natalensis* (gram-positive bacterium) [9]. Due to its broad spectrum of fungistatic activity and strong antifungal potency and enhanced corneal penetration, it is regarded as the front-line drug in the treatment of FK [10–12]. It was first isolated from fermented broth from a South African soil sample of *Streptomyces natalensis* by Gist-brocades Research Laboratories (The Netherlands) in 1955. *Streptomyces natalensis* and *Streptomyces gilvosporeus* are used for fermentation for industrial production [13, 14].

Nat is a BCS class II with an aqueous solubility of 30–50 mg/l at physiologic pH and is given as a suspension. It is more readily soluble in highly acidic and alkaline solutions but undergoes faster degradation under highly acidic and alkaline conditions [15]. It is a high-molecular-weight molecule (665.733 g/mol) with limited corneal permeation. It is prone to photo-oxidation and photodegradation upon exposure to heat, light, oxidants, heavy metals, extreme pH values and temperatures > 100 °C [16] attributed to chromophore cleavage caused by light absorption [17].

Despite the difficulties in using Nat to treat ocular fungal infections, it is still recommended in clinical practice for the superficial treatment of ophthalmic fungal infections. This may be attributed to the clinical efficacy or safety

profile of Nat eye drops in fungal infections, where Nat showed fewer side effects, lower resistance rates and lower ocular toxicity reactions compared to antifungals, such as amphotericin B and azoles [11, 18, 19]. To better understand the recent advancement in Nat ocular delivery, this review summarizes the various nanoformulations developed and studied with an aim to improve the corneal penetration and bioavailability of Nat, thereby reducing its dose and dose frequency. Furthermore, few reports of comparative studies conducted on amphotericin B, voriconazole and Nat are discussed, which highlight the superior efficacy of Nat in treating FK infection as well as the currently available marketed Nat suspension, and the challenges faced are briefly described. Finally, a brief description of Nat patents and future prospects are presented.

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

METHODS

A search was conducted on PubMed, Scopus and ScienceDirect using relevant keywords such as “natamycin,” “fungal keratitis,” “natamycin + ocular delivery” and “novel delivery system” and the marketed formulations of natamycin. The search identified various clinical trials and comparative case studies investigating the action of natamycin and various antifungal agents in the treatment of fungal keratitis, especially in the treatment of *Fusarium* keratitis cases, which are the most severe and least responsive cases of fungal keratitis. Furthermore, we examined an array of articles highlighting the various drawbacks of the currently commercially available natamycin 5% w/v suspension and current research studies on novel drug delivery strategies conducted to overcome the challenges and overall dose reduction and dosing frequency, consequently reducing the adverse effects associated with natamycin suspension. We extensively reviewed the literature and summarized it in this review article.

COMPARATIVE STUDIES OF NAT AGAINST OTHER ANTIFUNGAL DRUGS

Despite their potential antifungal activity, other antifungal medications, such as amphotericin B, flucytosine and fluconazole, have been reported to cause systemic toxicity [19]. Existing antifungal agents tend to fail to restore vision to its initial level after ocular infection [20]. Several comparative studies have been carried out with Nat, amphotericin B, voriconazole and other antifungal drugs against fungal species such as *Fusarium* and *Aspergillus* isolated from corneal infections to investigate which antifungal has the superior efficacy in treating a specific type of FK infection [21].

Prajna et al. compared the Mycotic Ulcer Topical Treatment Trial I (MUTT I) clinical outcomes of topical Nat and topical voriconazole therapy for filamentous fungal keratitis and found that Nat treatment showed significantly improved clinical and microbiologic outcome especially against *Fusarium* sp. and that perforation of the cornea and/or therapeutical corneal transplant was more prevalent in voriconazole-treated than Nat-treated individuals [11]. Similar results were observed when Sharma et al. compared the efficacy of topical 1% voriconazole and 5% Nat in treating fungal keratitis. Nat-treated cases showed greater improvement in vision especially in patients with *Fusarium* keratitis compared to those treated with voriconazole [22].

A similar study was reported by Spierer et al. comparing the in vitro antifungal activity of Nat (5%), amphotericin B (0.15%), fluconazole (0.2%) and voriconazole (1%) against *Candida* spp. isolates from human corneas. The results demonstrated that 100% of the samples showed growth inhibition upon treatment with Nat and amphotericin B compared to 19.6% and 85% overall inhibition rates of samples by fluconazole and voriconazole, respectively. For *Candida* keratitis, Nat and amphotericin B are the drugs of choice as voriconazole requires a higher concentration to inhibit *Candida* for the treatment of *Candida* keratitis [12].

Several researchers have investigated the minimum inhibitory concentrations (MICs) of different antifungals against different FKs causing fungal species (sp.). Cordeiro et al. investigated the antifungal potency of amphotericin B and observed that higher concentrations of amphotericin B (0.0625–4 µg/ml) were required to inhibit *Candida* sp. and about 19 species of *Candida* were resistant to it (MIC > 1 µg/ml) [23]. Similarly, a study by Pfaller et al. on invasive candidiasis patients in phase III clinical trials suggested that most *Candida* sp. growth can be inhibited by 0.25–0.5 µg/ml voriconazole; however, few isolates were resistant and required > 1 µg/ml voriconazole [24]. Nat has demonstrated variable MIC against *Candida* ranging from 1–2 µg/ml for *C. albicans* [25, 26] and may be as high as 150 µg/ml for *C. parasilopsis* as reported in the study by Salvosa et al. [27].

An in vitro antifungal study by Córdoba et al. against 29 *Fusarium* isolates suggested that MIC of amphotericin B and voriconazole ranges from 0.5 to > 16 µg/ml and 2–16 µg/ml, respectively [28], while that of Nat was found to be 4–8 µg/ml against 20 strains of *Fusarium* sp. [29]. A similar study carried out by Arikan et al. against 82 *Aspergillus* sp. found amphotericin B and voriconazole MICs to be 0.5 to 4 µg/ml and 0.125–2 µg/ml, respectively [30], whereas Nat exhibited an MIC range of 5–40 µg/ml against 191 sp. of *Aspergillus* [31]. Based on different studies, it can be concluded that voriconazole exhibits a narrower range of MIC with no remarkable difference among the three different fungal sp. and showed the highest MIC range for the *Fusarium* sp. Nat exhibits a higher and variable MIC range for the different fungal species but has excellent efficacy against

Fusarium, which is the major causative fungus of FK. Table 1 summarizes the MIC of Nat, amphotericin B and voriconazole against three different fungal species.

CURRENT MARKETED PRODUCTS AND THEIR CHALLENGES

Nat is the only topically administered ocular antifungal drug approved by the US FDA and is commercially available as a 5% w/v ophthalmic suspension [20, 32, 33]. Some of the commercially available Nat eye drops are provided in Table 2. According to a global survey, 80% of ophthalmologists treating FK propose that current medications are only moderately efficient in treating corneal fungal infections [34]. Reports suggest that fungal infections can be resolved with topical therapy in only 7.6% of patients, while 92.4% require surgical intervention [35]. Another disadvantage of existing topical treatments is that they must be administered numerous times a day over a long period, increasing the patient's treatment expense [7]. Nat suspension therapy is associated with poor corneal retention attributed to drug dilution and increased tear fluid turnover or due to nasolacrimal drainage and non-specific absorption [36, 37] with only about < 5% of the drug reaching the intended ocular tissue. A longer duration of therapy (4–6 weeks) is required to achieve optimal drug concentrations and to treat the infected ocular site, which is achieved by a high dosing frequency (1 drop every 1–2 h for first 3–4 days followed by 6–8 times daily for 14–21 days) [38]. The higher dosing frequency may cause patient non-compliance or even hospitalization [39–41]. The ocular anatomical

Table 1 MIC of natamycin, amphotericin B and voriconazole against three different fungal species

Fungal species	MIC (µg/ml)		
	Natamycin	Amphotericin B	Voriconazole
<i>Fusarium</i> sp.	4 to 8	0.5 to > 16	2 to 16
<i>Aspergillus</i> sp.	5 to 40	0.5 to 4	0.125 to 2
<i>Candida</i> sp.	1 to 2 (variable)	0.06 to 4	0.25 to 0.5

Table 2 Marketed natamycin eye drops

Sr. no.	Brand name	Manufacturer	Dosage	Side effects/contraindications
	Natacyn [®] (5% w/v)	Alcon Laboratories, Inc	5% solution for 1–2 days is instilled as 1 drop every 1–2 h; after 3–4 days instill 1 drop 6–8 times per day	Eye discomfort, irritation, pain, swelling, foreign body sensation, changes in vision, tingling and tearing
	Natacin [®] (5% w/v)	Entod Pharmaceuticals Ltd	Duration: 2–3 weeks	Contraindicated in patients with hypersensitivity
	Natamet [®] (5%/3 ml)	Sun Pharmaceuticals Industries Ltd. (Milmet)		
	Pimafusin [®] (5%/3 ml)	Elder Pharmaceuticals Pvt. Ltd		
	Myconat [®] (0.5%/ml)	Ophtho Remedies Pvt. Ltd		
	Nata Aid [®] (5%/5 ml)	Nri Vision Care Pvt. Ltd		
	Natadrops [®] (0.5% w/v)	Cipla Limited		
	Natam [®] (5%/1 ml)	Mepharma Alna Biotech		
	Natoptic [®] (0.5% w/v)	FDC Limited (Lumina)		
	Natoptic [®] (5% /1 ml)	FDC Limited (Lumina)		

layers restrict drug penetration leading to poor absorption, and the high tissue binding potential of Nat leads to low bioavailability (< 5%) [42, 43] and is not recommended for treating deep stromal fungal infections. The long dosing schedule of Nat suspension with shorter residence time at the site of action causes failure of therapy and an increase in resistance to FK [32, 44]. The eye suspension is reported to cause several adverse effects such as irritation, discomfort and eye pain, allergic reactions (not significant), chest pain, eye hyperemia and

edema, corneal opacity, changes in vision, dyspnea, paresthesia, foreign particle sensation and tearing caused by longer duration of therapy [38].

NOVEL FORMULATION STRATEGIES FOR NAT OCULAR DELIVERY

Despite its widespread use, formulating Nat formulations that provide both targeted and efficient antifungal action is arduous [9]. Owing

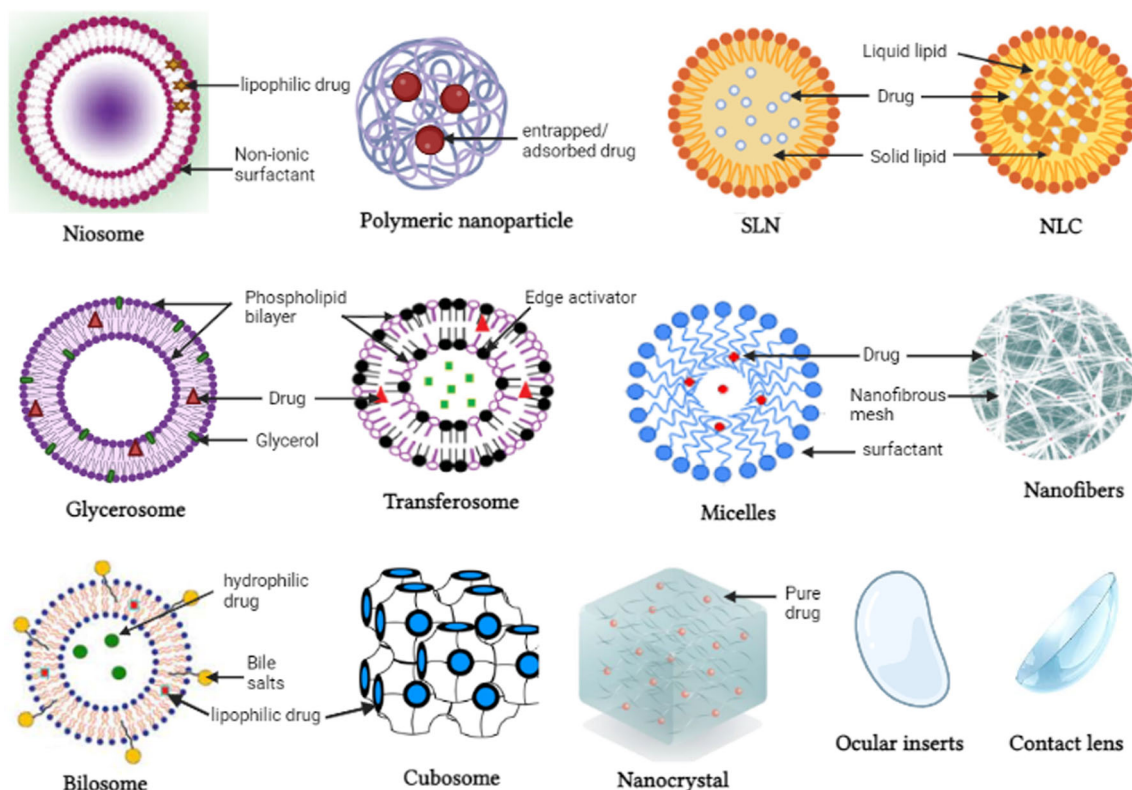


Fig. 1 Novel drug delivery systems have been used to enhance topical natamycin ocular delivery

to the presence of a complicated anatomy and many static and dynamic barriers, achieving an effective drug concentration in the eye via the topical route is complicated. Low ocular bioavailability (< 5%) is attributed to the rapid clearance from the precorneal region and minimal corneal permeability of conventional drug formulations. Therefore, the development of a drug delivery system with improved precorneal retention and sustained drug release over long periods would be a potential strategy for therapeutic interventions for ocular surface infections [41].

The administration of conventional eye drops is convenient but leads to suboptimal management of the disease. Novel topical drug delivery techniques can enable better corneal permeability and ocular retention, thereby maintaining the Nat MIC in the lacrimal fluid, which helps to treat the infection with minimal to no risk of development of resistance and recurrence [45]. With the advancement of nanomedicine and nanotechnology, various novel therapeutic approaches and strategies for

delivering drugs to their intended targets have been proposed [46]. Several researchers around the globe have explored novel approaches to Nat ocular administration such as nanoparticles, niosomes, nanomicelles, cell-penetrating peptides, nanocrystals, nanofibers, etc., to improve the ocular bioavailability and efficacy of Nat against FK. In this review we have briefly reviewed a few studies done by some researchers and Table 3 summarizes the advantages, drawbacks and stage of experimentation of the various novel natamycin delivery systems (Fig. 1).

Colloidal Nanocarriers

Polymeric Nanoparticles

Nanoparticles (NPs) are mucoadhesive, biodegradable and composed of drug molecules dissolved or encapsulated in a matrix of natural or synthetic polymers (polycaprolactone, albumin, sodium alginate and chitosan). Nanoparticles are promising candidates because of their small size (50–400 nm), which results in

reduced eye irritation and improved permeability owing to the longer residence time of the drug at the ocular surface. It provides sustained drug release by acting as a drug reservoir, leading to reduced dose and dosing frequency [47–49].

Chandasana et al. designed a Nat-encapsulated 1% and 5% poly-D-glucosamine (PDG) functionalized polycaprolactone (PCL) nanoparticle suspension for prolonged corneal targeted drug delivery. The 5% PGD-PCL Nat-loaded NPs showed significantly higher C_{max}/MIC_{90} (\sim tenfold) and $AUC_{(0-10)}/MIC$ (\sim 125 fold) compared to Natamet[®]. Amino groups of PDG (cationic polysaccharide) have a higher binding affinity to anionic mucosal sialic acid residues in the corneal epithelium, which aids in improved precorneal retention at the ocular surface. Furthermore, PDG is reported to have fungicidal activity and ocular biocompatibility and is known to enhance the paracellular transport of drugs [50].

Polymeric NPs have the potential to sustain drug release and improve pharmacokinetics compared with conventional eye drops. Nat encapsulated lecithin/chitosan (LC/CS) NPs showed sustained drug release for up to 7 h compared to 2 h with Natamet[®]. The $AUC_{(0-\infty)}$ of the drug increased by 1.47-fold, while the clearance of Nat decreased by 7.4-fold compared to the Natamet[®] suspension. CS is a cationic polyelectrolyte that has a strong affinity for the anionic corneal surface, resulting in an increased retention time. CS offers ophthalmic biocompatibility, non-toxicity, biodegradability and mucoadhesion [10] whereas LC can enhance the lipophilic drug loading and sustained drug release because of its lipophilic nature [51]. When choosing a treatment protocol and dosage regimen for an antifungal drug like NAT that exhibits concentration-dependent killing, the PK/PD indices are acknowledged as a vital element.

Recently, Sha et al. developed a thermosensitive Nat-loaded tri-block polymer NP-polyoxamer gel complex and studied its safety, efficacy, penetrability, adhesion and antifungal activity in the treatment of FK. The tri-block polymer consisted of poly-lactic-co-glycolic acid, polyethylene glycol and poly-ethylene

imine, which provided greater penetrability, stability and utilization of the loaded drug. The micro-double dilution method was used to evaluate the antifungal efficacy of Nat-loaded tri-block polymer NPs, and they were compared with 5% marketed Nat suspension. The study showed that the MIC of the Nat NPs was 2 μ g/ml, which was half the MIC of the Nat solution (4 μ g/ml). The cytotoxicity test showed that the

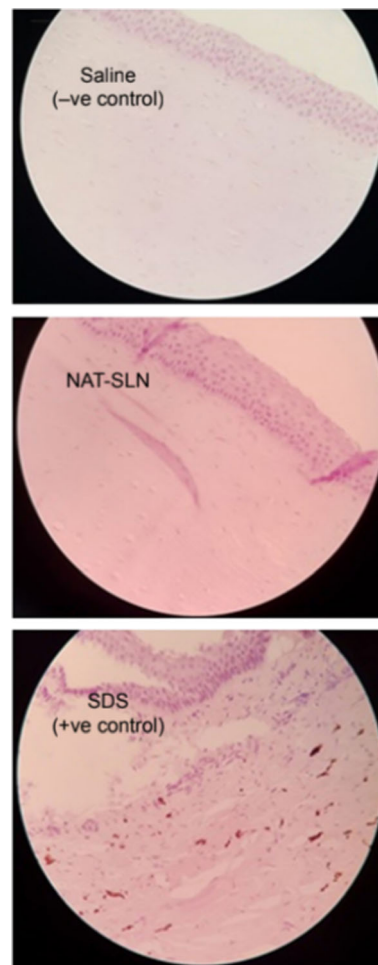


Fig. 2 Histopathologic images of corneal irritation studies performed on freshly excised goat corneas using the optimized NAT-SLN formula. The negative control and optimized NAT-SLN formulation showed no signs of morphologic damage to the epidermal corneal layers and no ocular irritation on the corneal surface structure compared to 0.1% SDS (sodium dodecyl sulfate), which showed significant damage to the epidermal layer of the cornea. Reproduced from [57] under Creative Commons Attribution License

Nat NPs had a higher cytotoxicity to human corneal epithelial (HCE-T) cells than the marketed suspension, which might be attributed to the cytotoxicity or inhibition of cell proliferation (rather than killing of cells) by the polymer block, and the cytotoxicity of both formulations was concentration dependent. The in vivo trans-corneal penetration study, using male New Zealand white rabbits, showed that 2.5 mg/ml Nat NP-thermosensitive gel formulation had a therapeutic effect similar to 50 mg/ml Nat suspension and showed sustained release, with improved penetrability and higher residence time, which effectively increased Nat content in the eye without causing epithelial injury and inflammatory cell infiltration [52].

Lipid Nanoparticles (NLC and SLN)

Lipidic nanoparticles are spherical, submicron, lipidic organic nanocarriers (50–1000 nm) with a biodegradable and biocompatible solid lipid or a mixture of solid and liquid lipid cores, which entrap lipophilic or hydrophobic drugs, and an exterior surfactant shell that may be sustained in the ocular tissue system [49].

Solid lipid nanoparticles (SLNs) constitute a drug that is encapsulated in a solid lipid matrix. SLNs are reported to be the most effective colloidal drug delivery systems compared to liposomes and polymeric nanoparticles because of their non-toxicity, protection of the drug from degradation, better stability, enhanced drug bioavailability and sustained drug delivery [53, 54] with lower production costs. Furthermore, SLNs have desirable characteristics, such as biocompatibility, larger surface area, prolonged drug release, reduced clearance, improved penetration across ocular barriers and chemical preservation of unstable drug molecules with higher drug inclusion properties [48, 55, 56].

Khames et al. prepared Nat SLN formulations that were optimized using Box-Behnken statistical design (three-level, three-factor). The optimized Nat SLN formulation showed an initial burst release within the first 2 h followed by a sustained drug release for 8 h when studied using the modified rotating paddle dialysis bag diffusion technique. The initial burst release might be attributed to the surface adsorption of

Nat and solubilization of the outer layer of the particle. Furthermore, Nat SLN significantly improved antifungal activity against *Aspergillus fumigatus* and *Candida albicans*, as the minimum inhibitory concentration against the fungal strains was lowered by 2.5-fold compared to the unformulated Nat when tested using modified Kirby-Bauer method and broth microdilution method [57]. The (Fig. 2) gives the histopathologic images of the corneal irritation studies conducted on the goat corneas.

Abdelmonem et al. formulated Nat SLNs (using lipids + Tween 80 or Pluronic F127) loaded in a mucoadhesive gel using Carbapol 940 and hydroxypropyl methylcellulose (HPMC) using a 2^3 factorial design, which showed sustained in vitro release of Nat from the gels for 6 h (dependent on polymer concentration) compared to the marketed product. An in vitro antifungal study was performed using agar cup diffusion method against *C. albicans*, which reported that Nat-SLN with 5% Pluronic F127 and 1:1 mixed lipid-loaded 4% HPMC gel had the maximum zone of inhibition compared to the other formulations and the marketed product and hence was further analyzed for in vivo antifungal study on rabbits. The histopathologic and clinical findings confirmed that the above formulation provided maximum curing efficacy against *Candida*-related FK compared to the marketed product with a controlled release of up to 8 h, which in turn reduced the dosing frequency, thereby improving patient compliance [58].

Nanostructured lipid carriers (NLCs) are second-generation SLNs that exist as solid lipid matrices at body or room temperature and are structurally composed of a unique mixture of solid (fat) and liquid (oil) lipids with a formless, uneven (less ordered) lattice arrangement [59, 60]. NLCs exhibit enhanced pre-ocular residence time, which improves penetration and bioavailability as well as stability on storage with low toxicity [61, 62]; in addition, they address the major disadvantages associated with SLN, such as poor drug loading and drug expulsion during storage [48].

Patil et al. were the first to fabricate Nat-loaded PEGylated NLCs using a Box-Behnken design and evaluate them against a marketed

formulation. The transcorneal penetration (across isolated rabbit corneas) of 0.3% Nat-loaded PEGylated NLCs was ~ twofold higher than that of Nat-NLCs and ~ sevenfold higher than that of Natacyn®. An in vivo distribution study showed that PEGylated NLCs had a ~ twofold higher Nat concentration in the cornea and iris-ciliary body (ICB) compared to diluted Natacyn® (0.3%), suggesting improved corneal permeation of NLCs compared to Nat suspension [63].

Vesicular Systems

Niosomes

Niosomes are novel vesicular carrier systems (10–100 nm) [64] with a closed bilayer structure formed by the self-assembly of a non-ionic surfactant, where the central aqueous core entraps the hydrophilic drugs, while the lipophilic drug partitions between the bilayer hydrophobic tails [65]. Niosomes offer several advantages over liposomes, such as higher permeation potential, improved bioavailability, better chemical stability, controlled release characteristics with delayed clearance, better biocompatibility and biodegradability, and non-immunogenicity, and they are reported to have fewer side effects than liposomes in vivo [66–68].

El-Nabarawi et al. developed a dual-purpose Nat-loaded niosomal gel containing (0.5%) ketorolac tromethamine (KT) to improve Nat penetration through the cornea and decrease the inflammation caused by FK. The in vitro drug release of niosomal formulations analyzed using a membrane diffusion technique showed an initial burst release within the first 3 h attributed to diffusion and desorption of Nat present on the niosomal surface followed by sustained release (77.49% after 24 h) compared to Natacyn®, which showed 85.52% drug release after 8 h. The optimized niosomal formulation was then incorporated into a gel containing 0.5% KT. The Nat niosome-loaded gel showed HPMC-E4 and sodium carboxymethylcellulose (NaCMC) concentration-dependent drug release. The Nat gel analyzed for in vitro release using dialysis method showed prolonged release for up to 24 h, which

might be attributed to the highly viscous diffusion barriers formed by the hydrated polymer chains. The antifungal study was carried out using agar well diffusion technique against *C. albicans*, which demonstrated 8 µg/ml MIC for 4% Na.CMC niosomal gel compared to the 63 µg/ml MIC of 2% HPMC-E4; this may be attributed to the higher viscosity of the gel, which resulted in poor diffusion of Nat. The in vivo visual assessment showed no corneal irritation. Furthermore, histopathologic studies showed a significant and the highest reduction in the fungal load and inflammation of the cornea in the 4% Na.CMC niosomal gel-treated group compared to the 2% HPMC-E4 niosomal gel group. The combination (Nat-loaded niosomes/0.5% KT- 4% Na.CMC) enhanced the permeability through the cornea and ocular bioavailability, which reduced the dosing frequency, thereby reducing the systemic side effects, relieved the inflammation caused by *Candida* and improved patient compliance [65]. Similar results in ocular irritancy and ex vivo transcorneal permeation studies (Fig. 3) were obtained by Paradkar et al.

Verma et al. developed cationic mucoadhesive N-trimethylchitosan (TMC)-coated Nat-loaded niosomes. The TMC-coated Nat niosomes were reported to have a 1.5-fold higher AUC_(0–12) and 27 times higher MRT than the marketed formulation. It was also observed that niosomes containing a 2% w/v dose of Nat showed optimum efficacy and reduced toxicity at the nasolacrimal site while providing a sustained release for 12 h, which resulted in reduced dose and dosing frequency. The Nat-containing formulations exhibited time- and concentration-dependent killing of *C. albicans* when evaluated using a time-kill kinetics assay, which is conducted to assess the speed at which killing occurs at a given drug concentration [69].

Glycosomes

Glycosomes are modified liposomes, which are colloidal concentric bilayer structures composed of phospholipids, water, high amounts of glycerol (10–30% v/v) and cholesterol [71].

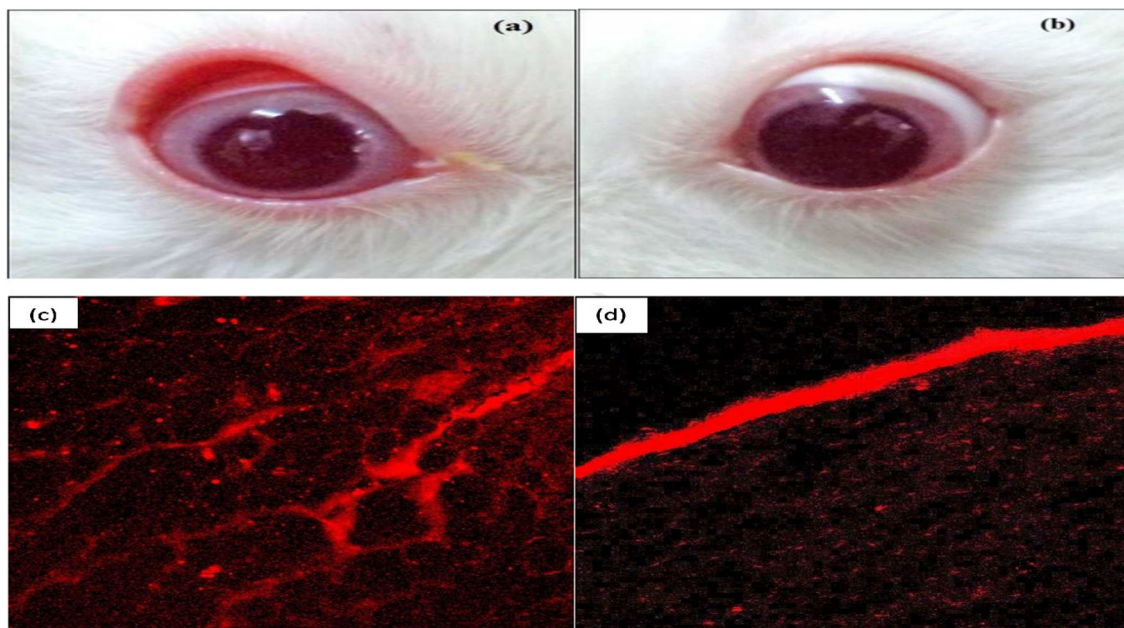


Fig. 3 Ocular irritancy study images of rabbit eyes: **a** untreated left eye; **b** optimized niosomal in situ gel formulation-treated right eye after exposure for 1 week. No symptoms of ocular irritation, ocular damage or any abnormal clinical signs of the cornea, iris or conjunctiva were observed. Images of the ex vivo transcorneal permeation study carried out using niosomes labeled with rhodamine B (fluorescent dye) and observed under a

confocal microscope; **c** the red dot indicates the internalization of niosomes into the cells across the cornea; **d** the corneal tissue surface-stained red with the dye showing bright red fluorescence. The niosomal formulation showed enhanced therapeutic effects owing to greater permeation across deeper layers of the cornea. Reproduced from [70] with permission from Elsevier

Glycerosomes are preferred over conventional liposomes owing to their higher stability and fluidity. Glycerol enhances the deformability index of glycerosomes, providing better penetration across the ocular barriers. Glycerosomes have been explored for topical and skin drug delivery and are reported to be safe and non-toxic [71–73].

Gupta et al. developed novel Nat glycerosome eye drops and studied their potential antifungal activity compared with conventional Nat liposomes and marketed Nat eye drops. Glycerosomes showed increased entrapment efficiency (80.84%) compared to conventional liposomes (59.5%). Furthermore, the Nat glycerosomes showed enhanced in vitro drug penetration (93.422%) and provided a controlled and better retention effect near the cornea compared to the conventional liposomes (57.6%) and Nat eye drop when performed

using the Franz diffusion cell method with a dialysis membrane. The ex vivo studies performed on excised goat corneas exhibited relatively higher corneal penetration by the glycerosomes (~ 80%) compared to the conventional liposomes (~ 75%) and the Nat eye drops (~ 20%). The superiority of glycerosomes over conventional liposomes was attributed to the large glycerol vesicles, which have a higher drug entrapping efficacy [74].

Bilosomes

Bilosomes are novel, closed, stabilized vesicles composed of nonionic amphiphilic surfactants in a bilayer that contain bile salt nanovesicular carriers and resemble niosomes [75, 76]. Bilosomes are highly flexible and ultra-deformable vesicles that help in drug penetration across the cornea, provide higher drug stability and have

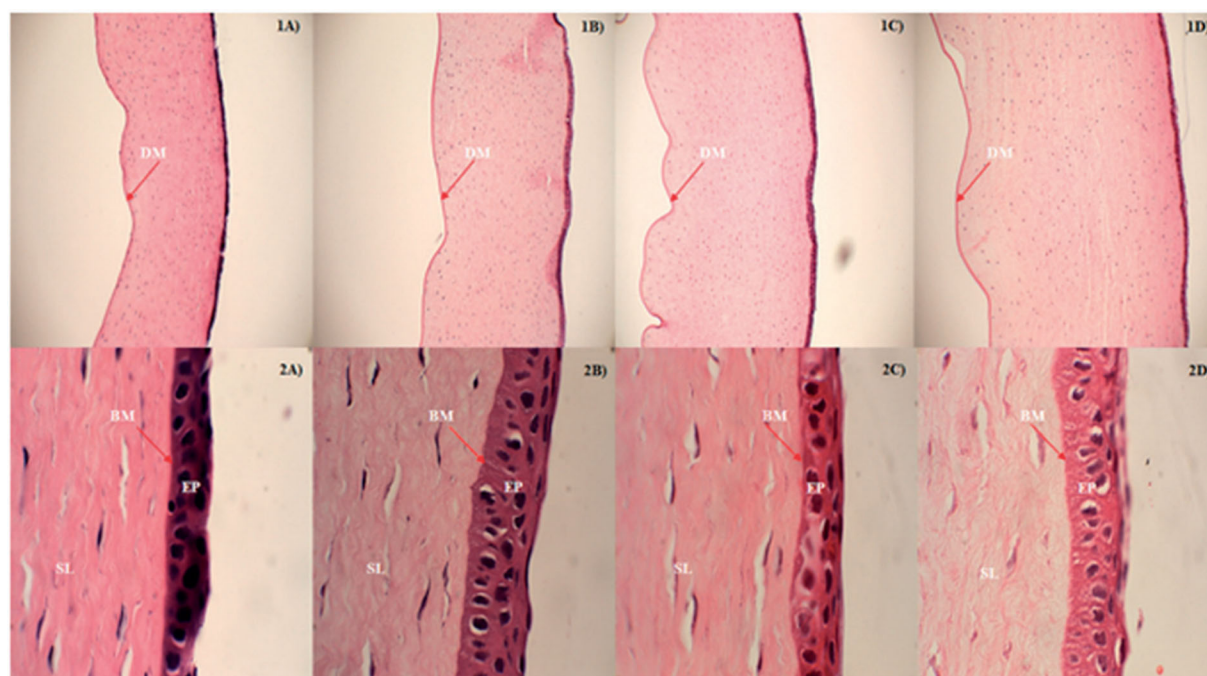


Fig. 4 Histologic images (10 × and 100 × magnification, respectively) of rabbit corneas exposed to **A** DPBS (2.5% w/v randomly methylated- β -cyclodextrin), **B** control, **C** natamycin bilosomes (NB 2) and **D** natamycin bilosome in situ gel (NBG 2) formulations. There were no signs of damage to the corneal structure in the rabbit eyes treated

with NB 2 and NBG 2, indicating corneal compatibility and safety, and the corneas were structurally similar to those treated with a control marketed suspension and DPBS. Reproduced from [78] under Creative Commons Attribution License

been extensively studied for improving drug delivery through oral and transdermal routes. Bilosomes offer a potential alternative to ocular drug delivery because of the presence of surfactants and bile salts that protect the drug against enzymatic degradation and also provide stability and higher permeation across ocular barriers owing to the nanosized vesicles [75, 77].

Janga et al. investigated Nat bilosome-loaded ion-sensitive in situ gels by using gellan gum and xanthum gum. Gellan gum in situ gel showed superior mucoadhesion and rapid sol-gel conversion compared to xanthum gum. In vitro cytotoxicity evaluated by methyl thiazolyl tetrazolium (MTT) assay and histology studies performed on rabbit corneas (Fig. 4) demonstrated the non-toxicity of gellan gum gel to corneal epithelial cells. The ex vivo study conducted on excised rabbit eyes suggested improved transcorneal flux by six- to ninefold compared to Natacyn[®]. The ocular disposition

studies suggested significantly higher Nat concentration in ocular tissues in the bilosome-loaded in situ hydrogel-treated group compared to Nat suspension after 6 h. Gellan gum and xanthum gum are anionic polysaccharide polymers specifically used in the study for their ion-sensitive gelling property. The formed hydrogels help in improving the pre-corneal residence time, and the shear thinning pseudoplastic behavior is important for eye comfort when blinking [78].

Transferosomes

Transferosomes are ultra-deformable modified liposomes prepared using an edge activator and a hydrophilic/amphiphilic single-chain surfactant, which impart superior transmembrane permeability. The edge activator regulates the vesicles according to the corneal epithelial

pores aiding in smooth permeation through the anatomical ocular barrier. The external stress causes the transferosome vesicles to change shape quickly, attributed to the edge activators, which diminishes the interfacial tension, increasing the carrier system's propensity to shrink [79–83].

Janga et al. developed Nat-loaded transferosomes incorporating ion-sensitive in situ gel using gellan gum. The corneal histologic studies have demonstrated biocompatibility, suggesting that transferosomes are a safer approach for ocular delivery. Nat-loaded transferosomes and in situ gel showed ~ 9- and ~ 16-fold improved corneal permeability compared to a drug suspension, respectively. The improved corneal permeation of in situ gel across the excised rabbit corneas (with a small portion of sclera) might be attributed to higher hydrophobic interactions or hydrogen or ionic bonding between gellan gum and cellular glycoproteins of gellan gum, imparting greater mucoadhesion [84].

Cubosomes

Cubosomes are unique bicontinuous cubic nanovesicles prepared using liquid-crystalline cubic clusters with high surface areas and microstructures. Cubosomes have the capacity to self-assemble in water to form 100–500-nm size range cubosomes with a structure comparable to that of honeycomb with internal aqueous channels [85, 86]. Cubosomes have the potential to encapsulate hydrophilic, hydrophobic and amphiphilic drug molecules with additional bioadhesive properties, sustained release effect and easier biodegradability by the enzymes, which enhances drug residence time at the corneal surface along with a reduction in dosing frequency [87, 88].

Hosny et al. investigated Nat cubosomes loaded with a pH-sensitive in situ gel using 1.5% Carbopol 934 to reduce the dose and dosing frequency and overcome the side effects associated with marketed eye drops. The in situ gel improved precorneal retention and showed a 3.3-fold enhancement in ex vivo Nat permeation (performed on excised corneas of albino

rabbits) compared to the 2% Nat marketed suspension and 5.2 fold compared to the Nat suspension. The in vivo ocular irritation study conducted on New Zealand white rabbits suggested that the developed cubosomal formulation was safer and less irritating compared to the marketed formulation [46].

Kazi et al. studied the use of Nat cubosome nanoparticles by implementing a 3^2 factorial design, aiming to improve corneal permeation. Cubosomes demonstrated an initial burst release of the drug, followed by sustained drug release for up to 8 h. The ex vivo corneal permeation studies performed on freshly excised goat corneas revealed higher apparent permeability (20.59×10^{-2} cm/h) and flux (5.46 mol/h) of the cubosomes, which reinforced improved Nat corneal permeation attributed to the nanosize and lipophilic structure of the cubosomes compared with the pure drug suspension. The Draize test demonstrated the non-irritant property of the cubosomes with a zero irritation score and no ocular damage [89].

Miscellaneous

Micelles

Micelles are self-assembled nanocarriers composed of a lipophilic core and hydrophilic shell composed of surfactants or amphiphilic copolymers. The lipophilic core aids in specifically entrapping lipophilic drugs, while the hydrophilic portion serves as a steric barrier that prevents aggregate formation and promotes solubility in aqueous or biologic media, such as lacrimal fluid. The micelles remain stable for a long time, resulting in delayed clearance, thereby providing tailored drug release by controlling their size, composition, functionality and overall morphology [90–93].

Guo et al. synthesized self-assembling mucoadhesive poly (ethylene glycol)-block-poly (glycidyl methacrylate) (PEG-b-PGMA) micelles to reduce the Nat dosing frequency for the treatment of fungal keratitis. The in vivo antifungal study concluded that the dosing frequency of Nat may be reduced from eight to three times/day owing to the sustained release of Nat from micelles with good clinical effects.

The Nat micelles were non-irritant and biocompatible, which was proved by the results of MTT assay, and imparted improved Nat solubility and corneal permeability. This was attributed to the mucoadhesive nature of the PEGylated shell of the micelles, which increased their retention time in the mucosal layer of the tear film. An in vitro release study carried out using the dialysis method showed an initial burst release due to the release of the loosely (untrapped) trapped free Nat on the micellar structure followed by a plateau phase [94].

Lorenzo-Veiga et al. developed polymeric nanomicelles using Soluplus and Pluronic P103 dispersions, with or without α -cyclodextrin (10% w/v), to improve aqueous solubility and enhance ocular penetration. Nat solubility was enhanced by 6-, 3.27- and 2.77-fold with Soluplus, Pluronic P103 and their mixed micelles, respectively. Vertical Franz diffusion cells were used to study in vitro drug release, and the results showed that Pluronic P103 (10%) micelles had the fastest drug release in 6 h with faster diffusion and a higher permeability coefficient, which was attributed to the smaller size and less compact structure of the micelles compared to mixed micelles and Soluplus. The poly(pseudo)rotaxanes (ternary complexes) system increased the viscosity of the micellar formulation, thereby increasing the residence time at the corneal surface and also causing a decrease in drug diffusion compared with the corresponding micelles. The ex vivo study conducted on excised bovine eyes using vertical diffusion Franz cells demonstrated that Soluplus-based poly(pseudo)rotaxanes improved the solubility of Nat in the aqueous layer surrounding the cornea leading to an increase in drug accumulation but showed the lowest permeability coefficient, attributed to the larger size of the encapsulating carrier and hindrance by the ocular tissue. The HET-CAM assay suggested that all the micellar formulations were non-irritant [95].

Cell-Penetrating Peptide (CPP)

CPPs, also known as protein translocation domains (PTD), membrane translocation sequences or Trojan horse peptides, are a family of peptides with generally 5–40 amino acid (aa)

sequences. CPPs can traverse human, plant and bacterial cell tissues and membranes via energy-dependent or -independent pathways, with no interaction with receptors. CPPs possess antimicrobial properties with low cytotoxicity and facilitate the cellular internalization of covalently or non-covalently bound drug molecule [96]. Since the discovery of TAT peptide as a cell-penetrating peptide in 1988, a slew of CPPs has been discovered, produced and studied for use in the treatment of many disorders [97]. Johnson and coworkers' peptide for ocular delivery (POD) was the first CPP formulated for ocular administration in 2008 [98]. KAI9803, RT001, RT002 and XG-102 are some of the CPP-conjugated therapeutics currently under clinical studies for the management of ocular diseases [96, 99].

Jain et al. was the first to conjugate Nat with Tat-dimer (Tat₂ and MTat₂), a CPP, to elucidate Nat uptake ability in corneal epithelial cells and antifungal activity. The study revealed an increase in Nat solubility of up to 100-fold in water and showed enhanced cellular penetration potential. A small concentration of Nat-conjugated CPPs (10 μ M) when assessed against *Fusarium solani* using a two-fold microbroth dilution assay showed that it completely inhibited the growth of *F. solani*, thereby exhibiting a twofold enhancement in antifungal activity compared to unconjugated Nat [7].

Rohira et al. conjugated Nat with Tat₂ and evaluated its tissue penetration potential and antifungal efficacy compared to the Nat (5% w/v) marketed formulation. The topically applied Nat-Tat₂ showed a five-fold higher ocular penetration and accumulation in aqueous and vitreous humor, which indicates improved Nat bioavailability compared to pure Nat. The cytokine study of Nat-Tat₂ using the Cytokine Bead Assay Kit showed a marked reduction in IL-6 while Tat₂ reduced levels of IL-6 and IL-1 β . For efficient antifungal activity against *Fusarium* sp. in vivo, the Nat-Tat₂ conjugation significantly reduced the Nat dose from 5% w/v (marketed formulation) to 0.330 mg ml⁻¹ (Nat-Tat₂ concentration equivalent to 100 \times 10⁻⁶ M) [100]. The (Fig. 5) gives the grade reduction and cumulative clinical scores pre-treatment and post-treatment using

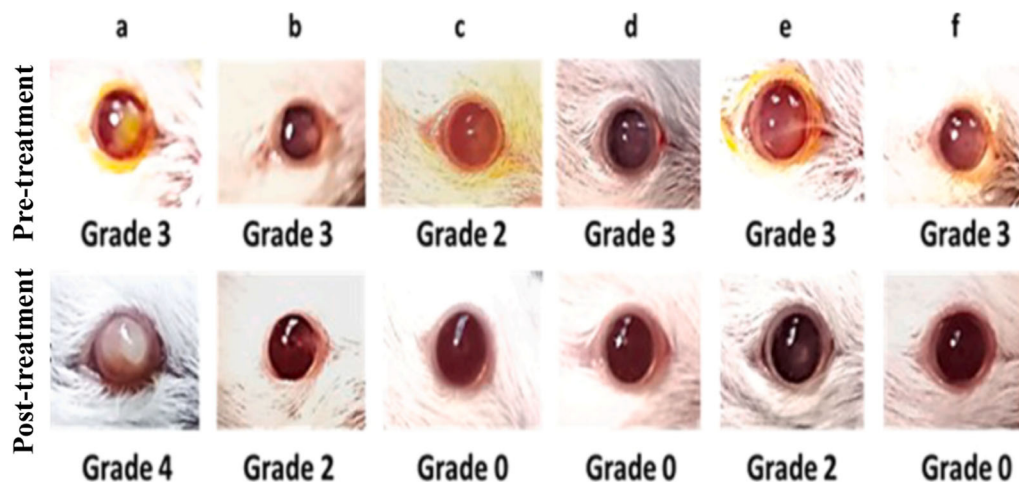


Fig. 5 Effect of PBS, 5% natamycin suspension, Tat₂-natamycin and Tat₂ on grade reduction and cumulative clinical scores pre-treatment (a–f) and post-treatment using slit-lamp biomicroscopy performed on New Zealand white rabbits. Representative images of grade reduction in each group after 6 days of treatment: **a** PBS ($n = 7$), **b** 5% natamycin suspension ($n = 8$), **c** Tat₂-natamycin (200×10^{-6} M) ($n = 8$), **d** Tat₂-natamycin (100×10^{-6} M) ($n = 9$), **e** Tat₂ (200×10^{-6} M) ($n = 7$) and **f** Tat₂ (100×10^{-6} M) ($n = 7$). After 6 days of treatment, the animals treated with 5% natamycin suspension showed accumulation of the drug around eyelids leading to discomfort and difficulty in opening the

eye, and animals with lower grades of fungal keratitis (grade 0–2) showed increased body weight and physical activity. About 44% and 38% of animals exhibited complete resolution of the disease with no vascularization or endo-exudates after being exposed to Tat₂-natamycin (100×10^{-6} M) and (200×10^{-6} M), respectively, compared to 5% natamycin suspension, while the animals treated with Tat₂ 200×10^{-6} M showed higher vascularization with about 29% and 14% showing complete resolution upon treating with 100×10^{-6} M and 200×10^{-6} M of Tat₂, respectively. Reproduced from [100] with permission from Elsevier

slit-lamp biomicroscopy performed on New Zealand white rabbits.

Nanocrystals

Nanocrystals are pure drug nanoparticles with sizes ranging from 1 to 1000 nm and have a higher drug-loading capacity than other nanocarriers, which aids in the efficient transport of drug molecules into cells. Nanocrystals provide higher saturation solubility, faster dissolution rate, higher mucoadhesion and enhanced saturation solubility, leading to a higher concentration gradient and resulting in a greater diffusive flux at the ocular surface. Furthermore, nanocrystals with 100% drug loading may result in longer retention at the ocular surface [101–103].

Das et al. synthesized Nat nanocrystal-loaded pH-sensitive in situ gels using Carbopol 940P

and HPMC EL 50 V by implementing the Box-Behnken design and response surface curve to obtain optimized Nat nanocrystals that show improved solubility and absorption of Nat. The in vitro release study revealed faster drug release from nanocrystals (87.56% after 2 h) than from the pure drug. The in vitro diffusion of the in situ gel showed sustained drug release compared to the marketed formulation attributed to the retardant effect of the gel, which aided in improving the ocular bioavailability of the gel. The ex vivo studies demonstrated higher corneal permeation of the in situ gel attributed to extended dissolution compared to the marketed formulation [104].

Nanofibers

Nanofibers are nanosized biodegradable polymeric drug carriers with large surface areas

obtained by electrospinning and sol-gel processes. They offer high drug-loading potential, increased drug encapsulation efficiency and co-administration ability. Fibers can also aid in drug transit across physiologic barriers, reducing premature drug release, targeted delivery and controlled drug release [105, 106].

Veras and Ritter et al. fabricated and characterized poly- ϵ -caprolactone (PCL) Nat nanofibers by electrospinning four formulations. Formulations with tetrahydrofuran (THF): *N,N*-dimethyl formamide (DMF) (4:1, v/v), polyethylene glycol (PEG 4000), and medium-chain triglycerides (MCT) dissolved in THF: chloroform (CHCl₃) (3:1 v/v) showed higher Nat loading in the PCL nanofibers, resulting in enhanced antifungal activity against eight different yeast and filamentous fungal isolates. These nanofibers showed low hemolytic damage (1.2–6.5%) when hemolysis assay was conducted on heparinized sheep blood as a sufficient amount of Nat was not freely available to damage cells [107].

Contact Lenses (CL)

Contact lenses (CLs) are ocular prosthetic devices that can provide a combination of vision correction and ocular therapeutics, as reported by Sedlacek in 1965 [108]. The post-lens tear film is a fluid layer that separates the CLs from the cornea. It has a clearance time of approximately 30 min, which provides a prolonged contact time with the cornea compared to the few minutes of conventional eye drops [109, 110]. It has been reported that about 50% of the drug released from the CLs can diffuse into the cornea, which is 35-fold more efficient than eye drops. The polymer of the contact lens acts as a barrier or reservoir and facilitates the sustained release of drugs for a prolonged period, which diminishes the need for multiple doses and provides enhanced bioavailability. As a topical delivery system, the drug-laden CLs reduce the systemic absorption and circulation of the drug, thereby reducing the systemic side effects [108]. The simple soaking technique provides poor loading attributed to the very small drug concentration gradient. Poly-

(cyclo)dextrins are suggested to enhance drug loading in CLs by increasing the concentration of the drug in the aqueous phase, which in turn may also increase distinct drug network interactions [111, 112].

Contact lenses offer the significant advantage of improved ocular bioavailability, from < 5% of eye drops to ~ 50%, which is attributed to improved precorneal retention. However, the drug uptake mechanism and release kinetics of conventional lenses and silicone-based lenses are not well understood. Phan et al. studied the *in vitro* uptake and release kinetics of Nat from the different commercially available conventional hydrogel lenses (CHL) and silicone hydrogel lenses (SiHL). Although both CHL and SiHL released clinically significant drug, they suffered from burst release, suggesting their non-suitability for commercial use for the management of fungal keratitis [41]. Extensive research needs to be directed towards the investigation of CL material to impart sustained drug release. Several researchers are exploring different techniques to address the poor drug loading and burst release from contact lens. The major method employed for drug loading in CLs is a simple soaking technique, which suffers from poor drug loading and burst release. To overcome the challenges, Phan et al. explored cyclodextrin functionalized contact lens material for both conventional hydrogel and silicone-based lenses. The study suggested CDs' concentration-based improved drug loading, and drug release from CDs functionalized methacrylated β -CD and methacrylated HP- β -CD CL material. Higher CD concentration may provide an unfavorable arrangement of CDs, limiting the binding of the drug with CDS [113].

Ocular Inserts

These can be defined as drug-loaded, thin, sterile, multilayered, solid or semi-solid consistency devices that are specifically designed (size and shape) to be placed in the lower or upper fornix or cornea [114, 115]. These ocular devices can be formulated using insoluble polymers or soluble natural materials and can be developed

Table 3 Advantages, drawbacks and stage of experimentation of various novel natamycin delivery systems

Novel delivery system	Advantages	Drawbacks	Stage of experiments	References
Polymeric nanoparticles	Biodegradable, reduced eye irritation, better tissue penetrability, longer residence time due to improved ocular surface adhesion with controlled drug release property and high drug-loading capacity	Systemic toxicity of drug may be caused by polymer degradation leading to stability issues and also the presence of residual organic solvents may cause toxicity	Ex vivo corneal permeation studies, histopathologic studies and in vivo transcorneal permeation studies	[121–123]
Solid lipid nanoparticles	Non-immunogenic, biocompatible, biodegradable, low physiologic toxicity, sustained drug release without impairing vision, improved corneal permeation, enhanced ocular bioavailability	Drug expulsion during storage, higher lipophilic drug carry capacity (lipidic core) than hydrophilic drugs	Ex vivo corneal permeation studies, histopathologic studies and in vivo ocular biodistribution studies	[124–126]
Nanostructured lipid carriers	Biocompatible having inert nature, lipid core prevents drug degradation, enhanced pre-ocular residence time which improves penetration and bioavailability, improved stability on storage with low toxicity	Shorter residence time than SLNs due to lower viscosity and lower occlusive effect than SLNs	In vitro transcorneal permeation study and in vivo ocular biodistribution studies	[115, 127, 128]
Niosomes	Non-immunogenic, biocompatible, biodegradable, good drug (hydrophilic and hydrophobic) loading capacity, lower systemic drainage, osmotically active systems that are stable and increase stability of entrapped drug and controlled drug release	Poorer drug-loading capacity than nanoparticles	In vivo ocular retention studies, histopathologic evaluation, in vivo antifungal evaluation study and determination of pharmacokinetic parameters as well as PK/PD indices and histopathologic studies	[129]

Table 3 continued

Novel delivery system	Advantages	Drawbacks	Stage of experiments	References
Glycerosomes	Biocompatible, non-toxic, increased drug entrapment with higher corneal penetration, high stability, improved corneal penetration	Lower viscosity and flexibility leading to poor penetration are seen in glycerosomes with < 20% glycerol while increased glycerol entrapment in the vesicle causes delayed drug release and increased particle size	In vitro <i>drug penetration study</i> and ex vivo corneal penetration studies	[72, 130]
Bilosomes	Enhanced corneal drug permeability, improved bioavailability, protect drug against enzymatic degradation	Limited research on long-term stability aspects	Corneal histology studies and ocular distribution studies	[131]
Transferosomes	Ultra-flexible vesicular systems that easily cross the physiologic barrier, biocompatible, high entrapment efficiency, controlled release of drug, improved corneal drug permeability and bioavailability	Undergoes oxidative degradation, high manufacturing cost and the purity of the natural phospholipids determines the reorganization property of transferosomes	Corneal histology studies, ex vivo corneal penetration studies and ocular distribution studies	[79, 80, 82, 83]
Cubosomes	Enhanced ocular retention, fuse corneal epithelium cell membrane and act as drug reservoir, prolonging corneal drug residence time, better corneal penetration and stability	Less extent of hydrophilic drug entrapment, drug leakage may occur during storage, particle growth may occur upon standing	Ex vivo corneal penetration studies and in vivo ocular irritation studies	[132, 133]
Micelles	Exhibit corneal mucoadhesion, improved drug-loading capacity with enhanced drug penetration and high aqueous stability, provide sustained release	Dissociate at a concentration below critical micelle concentration, and large-scale production is difficult	Ex vivo corneal and sclera permeability studies and in vivo ocular irritation and biodistribution studies	[134]

Table 3 continued

Novel delivery system	Advantages	Drawbacks	Stage of experiments	References
Cell-penetrating peptide	Translocate across cell membranes, cell-penetrating peptides have antimicrobial property	Chemically unstable and prone to degradation by enzymes (intracellular and extracellular) and require the development of highly sensitive analytical methods for detection	Establish antifungal activity using murine model and histopathology study	[135–137]
Nanocrystals	Higher drug-loading capacity than other nanocarriers, exhibit good adhesion to the cornea, which increases pericorneal residence and consequently leads to higher bioavailability	Particle size growth leads to instability of nanocrystals and poor IVIV correlation, not suitable for hydrophilic drugs	Ex vivo corneal penetration studies and in vitro release studies	[102, 103, 138]
Nanofibers	High drug-loading potential due to small diameter sizes and large surface areas and provide controlled drug release	Some production methods are not suited for commercial scale production, and advances in nanofiber characterization are needed	Hemolysis assay, anti-fungal studies and drug release assays	[139]
Contact lenses	Prolonged corneal contact time, acts as drug reservoir with sustained drug release, reduced systemic loss	Difficulty in application, potential risks in safety, difficulty in manufacturing, changes in optical properties and oxygen permeability may occur at the time of production	In vitro analysis	[140–142]
Ocular inserts	Omits preservatives, reduced systemic absorption, longer corneal retention with enhanced bioavailability	Ocular inserts may be rigid, which may cause eye irritation and adversely affect vision. The application of some inserts and their attachment to the eye may be challenging	Ex vivo and in vitro transcorneal permeation studies and antimicrobial activity	[118]

Table 4 Details on natamycin patents

Name of inventors	Patent number	Patent title	Composition	Patented year
Bertus Noordam, Jacobus Stark, Ben R. De Haan, Hong Sheng Tan	US5552151	Stable natamycin suspensions	Thickening agents: (methylcellulose, carrageenan gum, Arabic gum, xanthan gum and their combinations) maintained in a pH range of 3–4.8 without the addition of any preservatives	September 3, 1996 (expired)
All India Institute of Medical Sciences, New Delhi	–	Novel topical natamycin Formulation for ocular antifungal therapy	Sterile water-soluble intrastromal natamycin (IS-NTM) of 10 µg/0.1 ml	Patent filed

as mucoadhesive or even soluble ocular drug inserts [116]. The major benefit of the inserts is having a high precorneal residence time, which provides a means to release the drug material at a preprogrammed rate [117] ultimately leading to higher bioavailability and prolonged drug activity. There is a decrease in systemic absorption, reduction in dosing frequency and opportunity to target the internal eye tissues. Issues like difficulty in application or eye irritation due to rigidity lead to a reduction in patient compliance [115, 118].

Bhandari et al. incorporated Nat solid dispersion into a polymeric ophthalmic film [polyvinyl alcohol (PVA), chitosan and ethyl cellulose] to increase the retention time at the site, provide sustained release and improve Nat bioavailability. In vitro permeation studies showed that with an increase in the concentration of the polymers, Nat release decreased, which may be attributed to the longer drug diffusion pathway. The ex vivo permeation study showed a lower drug permeation than the in vitro, perhaps due to the varying pore size of the cornea and some of the fatty tissues that prevent the drug permeation. An inhibition zone that was sustained for 24 h and maintained for up to 15 days was observed in the antimicrobial test (cup-let method), and the Draize eye irritation test revealed that the formulation was non-irritant [119].

Rajasekaran et al. designed controlled-release Nat polymeric ocular inserts using various proportions of Eudragit (L-100, S-100 and RL-100), hydroxypropyl methylcellulose phthalate and cellulose acetate phthalate with PEG-400 as a plasticizer. A formulation with 3% Eudragit RL-100 and 1% Eudragit L-100 showed minimum moisture absorption due to the hydrophobic molecular substitutions of Eudragit RL-100, which showed zero-order drug release with an extended Nat release period of 23 h [120].

PATENTS

Currently, there are two patents for the commercial production of Nat synthesized by fermentation using either of the two species, *Streptomyces natalensis* or *Streptomyces gilvosporeus*, and the processes described by both are similar [13, 26]. One patent was filed by inventors Bertus Noordam, Jacobus Stark, Ben R. De Haan and Hong Sheng Tan (patent number: US5552151) for stable natamycin suspensions, filed on April 11, 1995, and granted on September 3, 1996, which was assigned to Gist-brocades B.V. and granted by the US Patent and Trademark Office (USPTO). These are concentrated suspensions of Nat, which show good physical, chemical and microbial stability (> 14 days) and are suitable as stock suspensions

for synthesizing immersion liquids and coating emulsions for treating food, feed and agricultural products. The suspension contains thickening agents (methylcellulose, carrageenan gum, Arabic gum, xanthan gum and their combinations) and is maintained at a pH range of 3–4.8 without the addition of any preservation [143].

Currently, a patent on Natasol, a sterile water-soluble intrastromal natamycin (IS-NTM) of 10 µg/0.1 ml, soluble Nat prepared by the Ocular Pharmacology and Pharmacy Division of AIIMS, New Delhi, India, as an adjunct for topical therapy for recalcitrant filamentous fungal keratitis is pending [144, 145] summarized in Table 4.

CONCLUSION AND FUTURE PROSPECTS

The incidence of ocular fungal keratitis has been increasing, and conventional topical dosage forms offer poor management owing to the physiologic and anatomical barriers present in ocular delivery. Recent developments in Nat ocular delivery have increased the potential for efficient drug delivery over time, with increased drug retention times resulting in improved bioavailability and antifungal efficacy. Much research has been done on Nat delivery; however, no alternative dosage forms have reached the clinical setting. This may be attributed to economics (intensive investment in resources), physicochemical parameters, failure to scale up pilot studies (advanced process designs) and limited understanding of long-term toxicity. Most of the topical nanoformulations show an initial burst release, which may lead to increased nonspecific absorption. Another major challenge in marketing these formulations is the sterilization and counter-effect they have on the various physicochemical parameters of nanocarriers such as size, polydispersity index and stability; currently, none of the researchers have studied these effects on their formulation.

Furthermore, Nat is reported to undergo decomposition when exposed to ultraviolet or gamma radiation owing to the loss of the

tetraene structure. However, it is not inactivated by visible light unless there is a transfer of photoenergy. The photodegradation products contain a tetraene chromophore, which is different from the parent Nat. Most of the current research focuses on addressing the solubility and dose frequency issues of Nat, but more research into the drug's photostability is needed. For clinical approval of these formulations, an extensive study must be done on the stability, long-term toxicity and controlled drug release.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

Data Availability. Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

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