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Recent Advances of Nanocrystals for Occular Fungal Keratits

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Abstract
Scientists working on medication delivery formulations have a hurdle due to the eye's unique anatomy and makeup. The concern over the standard ocular formulation's lower bioavailability has sparked researchers' interest in creating other drug delivery systems. The bioavailability of medications can be improved by using formulations based on nanotechnology to get beyond the ocular barriers that exist in the eye. The development of numerous marketed medicines was facilitated by the introduction of nanocrystals, which helped address the issue of poorly soluble pharmaceuticals, particularly for oral and parenteral drug administration. Medication retention duration, bioavailability, and permeability across the corneal and conjunctival epithelium have all been demonstrated to increase with the use of nanocrystal-based formulations for ocular medication administration. We have emphasized the physiology of the eye and drug delivery hurdles in this review. The advantages and disadvantages of various ocular formulations based on nanotechnology are compared. Additionally, several techniques for creating nanocrystals using their unique technology are taken into account. This article emphasizes the usage of nanocrystals in overcoming a variety of ocular delivery problems while highlighting the benefits and applications for ocular formulation. We highlight the potential of using nanocrystals as a new approach to push the boundaries of ocular medication delivery.

Keywords: Bioavailability, Corneal permeation, Drug delivery, Drug nanocrystal, Nanotechnology.

Introduction

According to a World Health Organisation (WHO) report, someone worldwide loses their sight every minute and every five seconds. 1.3 billion People worldwide are estimated to have some sort of vision impairment, according to the international classification of diseases [1]. The patients' vision and quality of life are impacted by these ocular illnesses. There have been significant advancements in the management of ocular disorders [2]. The discovery of therapies for a number of ocular illnesses, including diabetic retinopathy, glaucoma, age-related macular degeneration (AMD), uveitis, and cataracts, has been the focus of intensive preclinical and clinical research during the past ten years [3]. Recent advancements in the clinical treatment of ophthalmic disorders include the use of gene therapy, anti-vascular endothelial growth factor drugs, laser eye surgery, and ocular sealants. [4–5]. Eye drops (solutions, suspensions, and emulsions), in situ gels, ocular inserts, contact lenses, punctum plugs, intraocular injections, and implants are just a few of the drug delivery techniques that have been studied for effective ocular medication administration [6]. Major obstacles to successful medication administration in the ocular area include the eye's structural characteristics and the physiological barrier [7]. Therapeutic delivery systems based on nanocarriers have been created to support targeted drug administration to the anterior and posterior portions of the eye. Though, scaling up and quality control issues are linked to the conversion of nanotechnology-based medicine delivery systems from the bench to the bedside [8].

The anatomical and physiological impediments to ocular medication distribution are the main topics of this review [9]. We also go through the drawbacks of standard formulations and alternative medication delivery methods [10]. Advanced nanocarriers have been proven to be successful in treating ocular illnesses by overcoming the drawbacks of existing methods [11]. A range of Nano medicines and their results are gathered in order to understand the role of nanocarriers in the treatment of eye disorders. [12]. Additionally, this paper discusses the existing difficulties in translating Nano medicine, such as its mass production and quality assurance issues [13]. "Drug nanocrystals" are defined as pure solid drug crystals with a size in the nanometer range that only include the active pharmaceutical ingredients (APIs) and essential stabilizers [14]. According to the Noyes- Whitney and Ostwald-Freundlich equations, nanocrystals with particle sizes in the nanometer range (100-1000nm) can increase the saturation solubility and dissolution velocity of poorly soluble drugs, improving drug absorption and bioavailability more effectively than the conventional formulation [15]. In the 1990s, the first drug nanocrystals, which are pure solid drug particles with a nano dimension, were created [16]. Water, water-reduced blends, or non-aqueous media can all be used as the dispersion medium [17]. Typically, steric effect or electrical repulsion are used by surfactants or polymers to stabilize nano-suspension [18]. A substance's physicochemical properties may change as it is scaled down to the nanoscale (below 1 m). According to the Kelvin equation, a substance's saturation solubility (Cs) increases with decreasing particle size [19]. The dissolving velocity (dc/dt) likewise increases when the specific surface area expands [20]. In order to solve the biopharmaceutical delivery problems of drugs that are not easily soluble, nanocrystal technology is widely used [21]. In addition to having higher saturation solubility and dissolution velocity, nanocrystals have stronger skin adhesion, which makes it easier to distribute them topically [22]. Therefore, incorporating the drug in nanocrystal form as a dissolving depot in dermal formulations (lotions, creams, or gels) may help skin penetration by maintaining both the formulation's dissolved drug concentration and the gradient of concentrations between the formulation, the drug, and the skin [23]. Nano-crystals are used to produce drugs with a high drug content and good water dispersibility [24]. Nano-crystals can be produced without the need for carriers, and the loading efficiency of the delivery system can rise to 50% or more. Because encapsulating excipients were not used in the manufacturing process for nano-crystals, side effects related to excipients may be fully avoided [25].

Table 1 List of nanocrystal-based formulations in market

Generic	Indication	Trade Name	Manufacturer
Rapamycin	Immunesuppressive	Rapamune	Wyeth [26]
Aprepitant	Anti-emetic	Emend	Merck [27]
Megestrol	Anti-anorexic	Megace ES	Par Pharmaceutical Companies [28]
Fenofi brate	Hypercholesterolemia	Triglide	Sciele Pharma Inc. [29]
Griseofulvin	Anti-fungal	Gris-Peg	Novartis [30]
Nabilone	Anti-emetic	Cesamet	Lilly [31]
Dexmethylphenidate hydrochloride	Anti-psychotic	Focalin XR	Novartis [32]
Diltiazem	Anti-angina	Herbesser	Mitsubishi Tanabe Pharma [33]
Theophylline	Bronchial dilation	Theodur	Mitsubishi Tanabe Pharma [34]
Fenofibrate	Hypercholesterolemia	Tridlide	Skye Pharma [35]
Silver	Anti-microbial	SILCRYST	Nucryst Pharmaceuticals [36]

Occular Drug Delivery System

Ophthalmic drugs have long been one of the most important and advanced fields of pharmaceutical technology [37]. The medication's limited absorption after application to the eyeball is primarily the cause of scientists' continuous intrigue with these pharmacological forms [38]. The complex structure of the eye [39], the cornea's small absorptive surface and low transparency, the lipophilicity, metabolism, and enzymolysis of the corneal epithelium, the drug's interaction with proteins in tear fluid [40], and the eye's defence mechanism [40], which includes tear production, blinking, and the flow of the substance through the nasocrimal duct [41], among other factors, all contribute to this.

Polymers were one of the first additions to conventional ophthalmic medication formulations [42], allowing for prolonged contact durations between the active ingredient and the ocular surface [43], increasing bioavailability [44]. Excipients were included in the formulation as a potential future technique to alter the bioavailability of the active ingredients in ophthalmic forms [45], which increased drug absorption into the eye [46]. Chelating agents, surfactants, and cyclodextrins are examples of these excipients, which are produced in inclusion complexes alongside the active ingredients [47]. As a result, poorly soluble drugs become more soluble, permeable, and bioavailable [48].

Fungal keratitis

Cilliary congestion, cellular infiltration, and corneal oedema are symptoms of the corneal inflammation known as keratitis [49]. It can spread both actively and passively [50]. Depending on where the illness originated (bacterial, fungal, viral, or acanthamoebic keratitis), several types of keratitis can manifest [51]. Mycotic corneal ulcers are most usually caused by the fungi Aspergillus flavus, Aspergillus fumigates, Aspergillus niger (most common), Candida, and Fusarium [52]. Fungi are to blame for more than half of all cases of fungal keratitis [53]. Fungus-induced keratitis is the main contributor to blindness and ocular morbidity [54]. Topical steroids, contact lenses, and trauma are a few risk factors [55]. Fungi can enter the stromal tissue through a break in the epithelial layer, develop there, invade the corneal stroma, cause tissue necrosis, and trigger an inflammatory response in the host [56]. Sadly, these organisms can pass through an undamaged Descemet's membrane to enter the anterior chamber [57]. As a result, getting rid of these viruses becomes more difficult [58]. Fungal keratitis, a serious condition that can be difficult to treat, is one of the main causes of ocular mycosis [59]. Suppurative corneal ulcers caused by fungus have become more common recently as a result of poor antibiotic and steroid treatment [60]. Fusarium species, which account for 41% of cases, are followed in frequency by Candida (14%), Curvularia (12%), and Aspergillus (12%) [61]. Even if the selection of antifungal drugs is currently restricted and frequently has undesirable clinical effects, fungal keratitis can still be treated [62]. Fungal ulcers are more prone to occur in patients with systemic or local immunosuppression, such as those with dry eyes, bulbous keratopathy, or postoperative cases of keratoplasty [63]. The spectrum of poor clinical results, poor corneal penetration, and limited stromal permeability [64].

Fungal keratitis is treated with the antifungal drug itraconazole [65]. It is a synthetic triazole medication that effectively fights Aspergillus, Curvularia, and Candida fungi [66]. After topical treatment, the commercial itraconazole ophthalmic preparation (Itral Jawa Pharmaceuticals, (India) Pvt. Ltd.), which contains 1% (w/v) itraconazole, causes precipitation in ocular tissue [67]. Due to the low corneal penetration and vision problems caused by conventional itraconazole dose forms such lacrimation, tear dilution, nasolacrimal discharge, and tear turnover [68]. It was determined to investigate itraconazole nanocrystals based on amphiphilic block copolymers for the treatment of fungal keratitis in order to circumvent the limitations of the conventional dose form [69].

Treatment plan

The various conditions that might result in fungal keratitis are included in Table 2, along with their numerous causes, suggested courses of treatment, and cutting-edge research on their consequences.

Table 2. Treatment plan to treat fungal keratitis

S. No.	Types of conditions involved	Effective route	Drug	References
1.	Endothelial toxicity	Intracameral	Voriconazole	Kernt et al., [70]
2.	Highly variable trough in aqueous humour.	Topical	Voriconazole	Michael et al., [71]
3.	Systemic infection present	Systemic antifungal therapy i.e., intravenous	Amphotericin B or Itraconazole	Thomas et al., [72]
4.	Ineffective in cases with deep stromal abscess,hence genrally effective against superficial infection.	Topical	Natamycin	Srinivasan et al., [73]
5.	After intravenous treatment, poor eye penetration and a large dose can seriously harm the kidneys.	Intravenous	Amphotericin B	Khoo et al., [74]
6.	Poor permeation occurs through intact epithelium in the cornea.	Topical	Amphotericin B	Srinivasan et al., [75]
7.	Given the lengthy nature of keratitis treatment, prolonged administration may cause impotence, gynecomastia, or baldness.	Oral	Ketoconazole	O'Day et al., [76]
8.	used as a second-line treatment for fungus keratitis when natamycin is ineffective	Topical	Miconazole	Foster et al., [77]
9.	Ocular irritation present	Topical	Econazole	Prajna et al., [78]
10.	Limited range of antifungal action	Topical	Fluconazole	Rao et al., [79]
11.	Poor corneal penetration occurs after systemic treatment and is frequently accompanied by gastrointestinal side effects.	Topical and Oral	Itraconazole	Prasad et al., [80]

Barriers in ocular drug delivery

Due to the eye's architecture and physiology, foreign objects cannot enter the eye at all [81]. The posterior segment of the eye, which is made up of the retina, choroid, and vitreous, is divided into two halves by the cornea, conjunctiva, sclera, and anterior uvea [82]. Despite being physically close to one another and having very different anatomical and physiological characteristics, the anterior and posterior regions of the eye cooperate and function separately when an ocular preparation is used [83]. In clinical practice, high drug doses are provided intravenously or intraviteral to treat the posterior part of the eye, while topical eye drops can be utilised to treat the front segment [84]. There are the

following anatomical barriers to medicine absorption [85]. The main causes of drug loss from the ocular surface include nasolacrimal drainage, systemic absorption of the medication from the conjunctival sac through the local blood capillaries, and non-productive drug removal [86]. A significant portion of medications are absorbed into the systemic circulation and lose their therapeutic efficacy [87].

Corneal barrier

Medication absorption into the eye from lachrymal fluid is limited by the corneal epithelium [88]. In terms of the trans corneal flux of medications, the cornea can be conceptualised as a trilaminate structure with the epithelium, stroma, and endothelium serving as the three main diffusion barriers [89]. The diffusional resistance provided by this tissue varies significantly, the epithelium exerts a barrier effect to hydrophilic medications, and the endothelium and epithelium contain 100 times more lipid material per unit mass of the stroma depending on the physicochemical properties of the drug entity [90]. 80–90% of the total mass of the cornea is made up of the stroma, which is composed of hydrated collagen. This functions as a diffusional barrier for highly lipophilic medicines [91].

Method For Preparation Of Drug Nanocrystals

Top-down and bottom-up technologies are the two different ways that nanocrystals are produced. Additionally, innovative techniques that combine the two main concepts have been created [92].

Bottom up technology

An established method for predicting precipitation that takes into account the fundamental concept of precipitation is the bottom-up approach [93]. The drug precipitates when a non-solvent is added after the medication has been dissolved in a solvent [94]. Important factors for this technology include the crystallinity and crystal size of the produced particles [95]. Managing the crystalline state of emerging particles is essential because amorphous nanocrystals are more soluble than their crystalline counterparts. Ostwald ripening will happen as a result of unchecked crystal development. Sono-crystallization, liquid jet precipitation, high gravity-controlled precipitation, multi-inlet vortex mixing, rapid expansion of supercritical solution (RESS), supercritical antisolvent, and evaporative precipitation into aqueous solution are a few examples of bottom-up technological variants [96]. The use of organic solvents in bottom-up methods, which must be eliminated from the finished product, is a major issue. The formulation could become chemically or physically unstable due to the remaining solvent. Additionally, because crystal development only occurs in one direction, generating physical instability, this approach yields nanocrystals with a needle-like form [97]. In order to avoid using organic solvents in this method, supercritical fluids are now used as a solvent or antisolvent. Evaporative precipitation into aqueous solutions is a new method that has been created for drugs that are soluble in water-immiscible solvents. Drug nanocrystals that are less than 100 nm in size can be produced using these approaches [98].

Top-down technology

This technique allows for the reduction of big drug crystals (in the micrometre range) to incredibly small sizes, ideally in the nanometer region. Drug nanocrystals are produced using high shear force techniques such media milling, micro fluidization, and high-pressure homogenization [99]. The most frequent method of administering the medication is in water with a stabilizer. Nanocrystals are created via media milling technology employing ball, pearl, and bead mills. The active pharmaceutical ingredient as well as one or more stabilisers are present in the dispersion medium, which is treated with grinding agents like pearl, bead, or balls [100]. The high-speed rotation of the grinding chamber, which produces significant impact owing to ball-ball and ball-wall collisions, reduces the drug

particle size from micrometre to nanoscale. The grinding chambers are made of stainless steel or a sturdy substitute for stainless steel [101]. For efficient and dependable grinding, the rotating speed of the grinding chamber is also essential. The grinding agent won't rotate as efficiently if the rotation speed is slowed, and the collision impact won't be powerful enough to reduce particle size [102]. Due to centrifugal force, the grinding agent will fill the chamber's edges quickly, resulting in inefficient grinding. Additionally, the rotating speed should be suitable for the kind of drug particle, the amount of grinding agent (about 30–50% of total mass), and the amount of time needed for adequate grinding. Glass, zirconium oxide, chromium, agate, and other specific polymers are used as grinding agents [103]. The size of the grinding agent is another crucial element that influences the milling process, although this takes a lot of time to process. The size of the produced nanocrystals will be lower if a smaller diameter grinding agent is used, which may be because there is a very small gap between the grinding agent and the substrate [104]. The right size and quantity of grinding agent must be selected in order to control the particle size of pharmaceutical nanocrystals. Surfactants and stabilisers are present in the dispersion medium used for milling, and they should be chosen based on their capacity to maintain viscosity and tolerate changes in temperature throughout the production process. How long milling will take is largely determined by the kind of drug nanocrystals being processed. (Firm or gentle) [105].

The size of the grinding agent is another crucial element that influences the milling procedure, although it takes a lot of time to process [106]. The size of the created nanocrystals will be lower if a smaller diameter grinding agent is used, which may be because there is a very small gap between the grinding agent and the substrate. Controlling the particle size of pharmaceutical nanocrystals requires careful consideration of the size and quantity of the grinding agent [107]. Surfactants and stabilisers are present in the dispersion medium used for milling, and they should be chosen based on their capacity to maintain viscosity and tolerate changes in temperature throughout the production process. The type of drug nanocrystals being processed determines how long grinding will take in the majority of cases. Drug particle size is reduced as a result of cavitation action when bubbles rupture [108].

Drug delivery mechanism from nanocrystals

Due to its beneficial properties, such as smaller particle size, improved solubility, and dissolution, nanocrystals have been investigated for the treatment of disorders that impact numerous skin layers. Three main processes allow the medicine to penetrate or pass through the skin when using topical formulations based on nanocrystals [109]. Drug nanocrystals interact better with biological membranes and have a mucoadhesive feature because they have a higher surface area than pure drug particles. According to the Noyes- Whitney equation [110], more surface area contributes to greater saturation solubility and dissolving rate. The medication is liberated from the nanocrystals after being administered topically and is then absorbed by diffusion [111]. The increase in drug nanocrystals' saturation solubility maintains a steady concentration gradient between the supersaturated solution and the target cell. Because of the large surface area of the nanocrystals, it acts as a reservoir, supplying continuous free medication through quicker dissolution. As a result, drug nanocrystals continue to release their contents over an extended period of time [112].

The medication is transported to the cell compartment or diffuses to the underlying tissues through endocytosis, the rapid ingestion of drug nanocrystals (less than 100 nm) by cells [113]. The gradient in drug concentration between the upper and lower layers of skin controls passive diffusion. In hair follicles, drug nanocrystals have been observed to accumulate, producing a depot of nanocrystal suspension that gradually enhances drug penetration into the neighbouring epidermal layers, according to studies. This improvement depends on concentration [114, 115].

Once the drug nanocrystals have diffused through the epidermal layers, endocytosis by the host cells may aid in their penetration further. Drug nanocrystals are now present in the host cell. Nanocrystals bigger than 100 nm are immediately taken up by host cells by macrophage phagocytosis. They travel through diffusion to the target area with the macrophage [116, 117].

Conclusion

Drug nanocrystals can be used instead of more conventional methods, such as the inclusion of surfactants and viscosity enhancers, to increase the ocular bioavailability of BCS class II and IV medicines. Industrial-scale manufacture of drug nanocrystals is made simpler by the arsenal of effective production technologies. Researchers are interested in creating these formulations to treat a variety of ocular disorders because of the advantages of using nanocrystals for ocular medication delivery. In conclusion, formulations based on nanocrystals may be used in the future for ocular administration. However, there are still a great deal of additional uses for nanocrystals that need research and proof of concept.

Consent for Publication

Not Applicable

Availability of Data and Materials

All data available in manuscript.

Ethical Approval and Consent to Participate

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