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Recent Patents on Ophthalmic Nanoformulations and Therapeutic Implications

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Abstract

Nanoformulations (NF) are widely explored as potential alternatives for traditional ophthalmic formulation approaches. The effective treatment of ocular diseases using conventional eye drops is often hampered by factors such as: physiological barriers, rapid elimination, protein binding, and enzymatic drug degradation. Combined, these factors are known to contribute to reduced ocular residence time and poor bioavailability. Recent research studies demonstrated that NF can significantly enhance the therapeutic efficacy and bioavailability of ocular drugs, compared to the established ophthalmic drug delivery strategies. The research studies resulted in a number of patent inventions, reporting a significant increase in therapeutic efficacy for various chronic ocular disease states of both the anterior and posterior ocular segments. This article reviews these patent disclosures in detail and emphasizes the therapeutic advantages conferred by the following nanoformulation approaches: Calcium Phosphate (CaP) nanoparticles, Liposomes, Nanoemulsions, Nanomicelles, and Hydrogels. The nanoformulation approaches were shown to enhance the ocular bioavailability by reducing the drugprotein binding, increasing the corneal resident time, enhancing the drug permeability and providing a sustained drug release. Further, the article discusses United States Food and Drug Administration (USFDA) approved ocular drugs employing nanotechnology and future developments.

It should be noted that, despite the potential therapeutic promise demonstrated by nanotechnology for ocular drug delivery, the bench to bed transition from patent inventions to marketed drug products has been insignificant. Majority of the discussed technologies are still in development

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

and testing phase for commercial viability. Further, studies are in progress to assess ocular tolerance and nanotoxicity for prolonged use of NF.

Keywords

Calcium phosphate nanoparticles; nanoemulsions; nanomicelles; ophthalmic bioavailability; ophthalmic nanoformulations

1. INTRODUCTION

Drug bioavailability of ophthalmic eye drop formulations is dependent on ocular barriers that restrict the drug permeability. The eye is a highly protective organ and its multiple physiological barriers make topical ophthalmic drug delivery a challenging area for formulation scientists [1]. It is reported that, ≤5% of drug in solution form is absorbed when administered topically [2]. Drug loss is primarily attributed to rapid drug elimination from the cul-de-sac due to lacrimation, blinking and tear turnover. In addition, factors such as lacrimal protein drug binding, drug metabolism by enzymes present in lacrimal fluid and poor corneal permeability, are found to be responsible for the poor drug bioavailability. Despite the challenges, a significant number of marketed ophthalmic formulations are available as topical eye formulations. The wide use is primarily due to better patient compliance and lower production costs.

Since topical eye drop solutions often have poor drug retention time, formulation scientists have explored various techniques to improve the corneal retention time. Enhancement of retention time on the cornea via viscosity builders has been widely explored; however this had a limited scope as liquid preparations were still subject to rapid clearance. Corneal drug penetration enhancers have also been explored, but due to the high sensitive nature of the corneal and conjunctival tissues, great caution was required to prevent toxicity. In recent years, hydrogel based ophthalmic formulations were extensively researched to extend the topical drug retention time. An example is sterile ophthalmic gel forming Solution (TIMOPTIC-XE®), manufactured by Merck Pharmaceuticals, USA [3]. The formulation contains anionic gellan gum that forms a gel when it comes in contact with cations. The major side-effect reported for the formulation was higher incidence of transient blurred vision. Tobradex-ST®, manufactured by Alcon Pharmaceuticals, USA was another innovative ophthalmic suspension that provides increased ocular retention and bioavailability of dexamethasone due to the gelling nature of xanthan gum [4]. As stated by Lallemand *et al*. (2012) [5], several excipients such as, “carbopol gels, cellulose derivatives, dextran, gelatin glycerin, polyethylene glycol, poloxamer 407, polysorbate 80, propylene glycol, polyvinyl alcohol, and polyvinyl pyrrolidone” have either viscosity enhancing or bioadhesive properties that can significantly improve the ocular retention time [5].

1.1. Efflux Transporters – Ophthalmic Drug Bioavailability

Efflux transporters were found to be primary barriers leading to poor drug absorption and bioavailability, primarily for anterior segment ocular drug delivery. A number of efflux transporters have been identified in the epithelial cells of various ocular tissues, in both

rabbits and humans. Notable among them are p-glycoprotein [also known as Multidrug Resistance Protein 1 (MDR1)], Multidrug Resistance-associated Proteins (MRP1, MPR2, MRP3, MRP4, MRP5, MRP6, MRP7, MRP8, MRP9, Si-MRP7), Breast Cancer Resistance Protein (BCRP) and Lung Resistance Protein (LRP). Corneal epithelial cells were found to express all the efflux transporters listed above in our research laboratory for the first time (Karla PK). The presence of many of these efflux transporters is not known to the scientific community prior to our research findings. Research efforts are in progress in our laboratory for an in-depth understanding of their clinical significance in ocular drug delivery [6–8].

2. OPHTHALMIC NANOFORMULATIONS

2.1. Nanoemulsions

Emulsions are fine dispersions of infinitesimal droplets of two immiscible liquids. By definition, nanoemulsions have dispersed phase in which the particle size is in the submicron or nanometer range. Nanoemulsions generally comprise of one or more amphiphilic lipid(s) or surfactants. Surfactants are molecules having a bipolar structure, containing at least one hydrophobic part and one hydrophilic part. The translucent appearance of nanoemulsions is attributed to the nanometer size (<100nm) of dispersed globules, which is obtained by high-pressure homogenization. Because of their globule size, nanoemulsions are thermodynamically unstable dispersions, often requiring a high concentration of surfactants to stabilize the formulation. This often leads to a sticky feel of the formulation and subsequent intolerance. In addition, phospholipids that are commonly used to stabilize a nanoemulsion formulation often results in yellowish appearance producing rancid odor after a short period of storage. A formulation that claims to overcome these disadvantages was developed and patented (US6335022B1) [9]. The invention describes a nanoemulsion utilizing oxyethylenated or nonoxyethylenated sorbitan fatty esters as surfactants. The inventors claim that the use of surfactants selected from oxyethylenated or non-oxyethylated sorbitan fatty esters, having a molecular weight >400 grams per mole, and are solid at temperatures ≤45°C can result in a stable formulation. In addition, inventors claimed that amphiphilic lipids selected from a group of alkaline salts of cholesterol results in a similar formulation stabilizing effect. The inventors claim that the optimized ophthalmic nanoemulsions can be utilized as effective delivery vehicles for anti-glaucoma, anti-viral, anti-allergic, and anti-inflammatory agents [9, 10]. Another ophthalmic nanoemulsion invention based on the surfactants selected from ethoxylated fatty ethers, ethoxylated fatty esters, and a mixture of both surfactants has been patented (US6375960B1) by the same inventor group [10]. In order to achieve improved bioavailability of ophthalmic formulations by increasing the residence time of drug on the cornea, a defined viscosity needs to be imparted to the ophthalmic formulations. A method of improving the viscosity is by increasing the fraction of dispersed oil phase. Alternatively, the viscosity can be increased by incorporation of water-soluble polymers [11]. The conclusion is that the water-soluble polymer will form a gel with the continuous aqueous phase, thereby increasing the viscosity. L'Alloret *et al.* reported that, "water soluble polymers such as hydroxypropyl cellulose, algal derivatives, natural gums, synthetic polymers and copolymers of carboxyvinyl acid (carbopols)", are widely employed to improve the viscosity of ophthalmic formulations. However, caution is advised in estimating the ideal concentrations of water

soluble polymers required to achieve the desired viscosity and yet maintain the transparency of ophthalmic formulations [12].

L'Alloret *et al.* developed and patented (US6998426B2) nanoemulsion formulations containing nonionic polymers [12]. The formulation was aimed to address the deficiency of earlier nanoemulsion developments described above, by modifying the viscosity of oil-in-water nanoemulsions without compromising the transparency. This invention describes an oil-in-water nanoemulsion containing either non-ionic surfactant, anionic surfactant or a blend of both. The polymers used were water-soluble non-ionic or neutral polymers selected from ethylene oxide, vinyl caprolactam, polyvinyl methyl ether, polyvinyl alcohol copolymers, a blend of these polymers and homopolymers,. The resulting nanoemulsion was transparent, stable and had oil globules with average particle size <100nm. The invention reported that the viscosity of nanoemulsion increased ~5-fold, when polymer concentration equivalent to 1% by weight was used [12].

Prostaglandins are oxygenated cyclic fatty acids of animal origin, formed primarily by the action of the enzyme cyclooxygenase. They are known to affect a wide range of physiological activities such as blood pressure, clotting, pain awareness and reproduction mechanisms. Some of these analogues have been found to be useful in ophthalmics as antiglaucoma agents. Examples of these agents include bimatoprost, latanoprost and travoprost, which are used in the treatment of ophthalmic hypertension (or) glaucoma [13, 14]. These agents however, have poor water solubility and are chemically unstable in aqueous solution. Various strategies suggested to overcome the challenges include formation of drug complexes with cyclodextrin and pH adjustment to improve solubility and stability. In addition, benzalkonium chloride (a non-ionic surfactant and a widely utilized ophthalmic antimicrobial preservative), is recommended to improve the aqueous solubility of prostaglandin analogues. This approach, however, resulted in positively charged nanoemulsions, which were often associated with ocular intolerability. In a recent patent disclosure (US8414904B2), Carli *et al.* described a nanoemulsion formulation made of an oil phase containing prostaglandin dispersed in an aqueous phase and stabilized by a combination of two (or) more non-ionic surfactants. This approach resulted in a chemically and physically stable emulsion with a neutral zeta potential (−1.9mV to 2.0mV). The formulation was found to be non-toxic in both *in vitro* and *in vivo* studies [15].

Cyclosporine A was widely used in the treatment of dry eye disease. However, poor solubility of cyclosporine A in aqueous medium results in drug precipitation, leading to ocular irritation. Improved formulations for topical delivery of cyclosporine have been reported in the patent disclosures US4649047 and US6582718 [16, 17]. Domb *et al.* also successfully developed aqueous formulations containing 0.01 to 10 mg/ml of cyclosporine A [18, 19].

At physiological pH, the corneal barrier has a net negative charge. Hence, a positively charged ophthalmic formulation as a strategy to prolong the drug residence time has been explored in various patents. United States patent No. 6007826 [20] describes an oil-in-water emulsion of colloidal drug particles bounded by interfacial film made up of surface active agents / lipids having positively charged polar groups. This resulted in a cationic oil-in-

water formulation having a positive zeta potential. In another patent disclosure (6656460), the inventors described a novel formulation strategy for treating the dry eye disease. The formulation is an oleylamine or stearylamine cationic emulsion with phospholipids, poloxamer as an emulsifying agent and benzalkonium chloride as an antiseptic agent. The novel cationic emulsion has been utilized for successful formulation of cyclosporine A, tacrolimus and sirolimus [21]. Another cationic oil-in-water emulsion has also been developed utilizing low concentrations of cationic agents (cetalkonium chloride, Benzalkonium chloride, oleylamine) and nonionic surfactants (poloxamer, tyloxapol). The resulting formulations had a positive zeta potential, an average particle size of 100–200 nm and improved stability. The methodology detailed in the patent disclosure (US8298568) was utilized to formulate a sirolimus nanoemulsion with improved toxicity profile when tested in a rabbit animal model [22].

Kuno and colleagues from Novagali Pharma S.A., France developed a cationic emulsion based on electrostatic attraction between the drug-loaded positively charged oil droplets and the negatively charged ocular surface [24]. The reported technology offered improved solubility and absorption of lipophilic drugs. This innovative formulation was manufactured in a three-step process. The first step involved phase mixing (oil and aqueous phases) with magnetic stirring at 100 rpm, followed by high shear mixing at 16,000 rpm. This resulted in an emulsion having oil droplets of particle size of 1 μm. To achieve the submicron size (150–200 nm), the emulsion was subjected to a high pressure homogenization at 1,000 bars at 4°C. Further, the patent describes using cetalkonium chloride (CKC) and benzalkonium chloride (BAK) as cationic agents to design a novel cationic nano vector. CKC is a highly lipophilic (log p = 9.5) component of BAK. It is used in oil phase to achieve a higher zeta potential on surface of the oil droplets. The advantages of utilizing BAK and CKC as cationic surfactants have also been patented by various United States and European inventors [25 – 27].

2.2. Soft Contact Lenses

Soft contact lenses have been employed in ocular drug delivery to prevent drug loss during administration, reduce systemic side effects and improve the efficacy. Chauhan *et al.* [28] invented soft hydrogel contact lenses loaded with various ocular drugs. The contact lens served as a matrix for the drug nanoparticles. The particle-loaded lens was clear and did not hinder vision due to the small size of nanoparticles. The inventors reported that the size range of drug-laden nanoparticles was ~50 nm to ~200 nm. The soft hydrogel lens was prepared from poly 2-hydroxyethyl methacrylate p-(HEMA). The drug was released from the lens by diffusion from the particles of lens matrix. The lens increased the drug retention time and drug permeation across the cornea, provided sustained drug release and minimized the systemic absorption via nasolacrimal sac. The inventors successfully demonstrated prolonged delivery of glaucoma drug (Timolol) with the use of the novel hydrogel lens [28].

2.3. Nanomicelles

Nanomicelles are self-assembling nanosized (10 to 100 nm) colloidal dispersions with a hydrophobic core and hydrophilic shell. Nanomicelles solubilize hydrophobic drugs within their hydrophobic core. The corona of the nanomicelles consists of hydrophilic chains that

extend outward, resulting in a clear aqueous formulation [29]. Mitra *et al.* [29] developed Rapamycin and Corticosteroid loaded nanomicellar formulation. Rapamycin has poor aqueous solubility (2.6μg/mL). The invention by Mitra reported that nanomicellar formulation significantly improved the solubility of rapamycin to 2 mg/mL (~1000 fold). The particle size of nanomicelles was ~25 nm. Rabbit animal model experiments on rapamycin nanomicellar eye drops revealed a noticeably higher concentration of the drug in the choroid/retina (~360 ng/g) and negligible concentrations in the aqueous humor, lens and vitreous humor. These studies demonstrated that nanomicellar formulation enabled drug delivery to the posterior segment with topical eye drop formulation [29].

Gupta *et al.* [30] developed polymeric micelles with copolymer of *N*-isopropylacrylamide, vinyl pyrrolidone and acrylic acid having cross-linkage with *N, N*-methylene *bis*acrylamide loaded ketorolac. The researchers reported significantly improved corneal permeation of ketorolac and anti-inflammatory activity compared to aqueous suspension of the drug. The positive outcome was attributed to the particle size (<50nm diameter) of the polymeric micelles and their mucoadhesive properties. A novel nanomicellar formulation of Voclosporin, a calcineurin inhibitor, has been developed for the treatment of dry eye syndrome. Mixed nanomicelles are manufactured from a combination of two non-ionic surfactants, d-a-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS) co-stabilized with octyl phenol ethoxylate (octoxynol-40) in a defined ratio. The resulting micelles were in nanometer size range, and the resulting aqueous solution was homogeneous and clear, making them an ideal ophthalmic drug delivery vehicle [29, 31, 32].

2.4. Nanoparticles

Nanoparticles with small molecule drugs as well as proteins, peptides and nucleic acids have been developed for targeted delivery. The colloidal formulation systems widely incorporated polymeric nanoparticles for ophthalmic delivery. Recently, Alexis *et al*. [33] invented stealth nanoparticles made up of polymeric matrix with a complex of phospholipid bound-PEG, PLGA and therapeutic agent (US20090074828A1). Ravi N *et al*. invented reversible hydrogel system containing nanoparticles and nanospheres. This patent (WO2012091278A2) describes a dilute solution of copolymers converting into hydrogels employing the mechanisms of oxidation/reduction, light intensity and mechanical stress as triggers. Nanocomposites were prepared by combining hydrogel and nanoparticles and *in situ* gel was formed upon injection into the eye for posterior segment drug delivery [34].

In a recent patent disclosure, Ketelson *et al.* [35] described the manufacturing of nanoparticles from inorganic materials. The inorganic nanoparticles were claimed to be useful in preventing (or) reducing the uptake of biocides from ophthalmic compositions by contact lenses. The inventors further demonstrated that the uptake of biocides by contact lenses is minimized without compromising the microbiological activity with the use of inorganic nanoparticles.

Calcium phosphate (CaP) nanoparticles have shown great applications in drug delivery systems due to biocompatibility (non-toxic degradation products constitute the inorganic part of human hard tissue) [36]. As inorganic CaP nanoparticles were found to be chemically stable, they were found to ensure the maintenance of desired pharmacological action [36].

The successful development calcium phosphate particle as an ocular drug delivery system was demonstrated in patent disclosure (WO2004050065A1) by Biosante Pharmaceuticals [37]. Studies have shown that ocular drugs can interact and bind with pigments, leading to decreased ocular bioavailability [38, 39]. CaP nanoparticles were shown to reduce drug binding with pigments and increase the bioavailability [37]. Chu *et al*. demonstrated that employing 7-hydroxy-2-dipropyl-aminotetralin (7-OH-DPAT) and CaP nanoparticles improved the intraocular pressure (IOP) and aqueous humor flow rate in both pigmented and non-pigmented rabbits [40]. D_2 and D_3 receptor agonists are proven anti-glaucoma agents for reducing the intraocular pressure (IOP). Chu *et al.* examined the relative effect of D_2/D_3 receptor agonist, 7-OH-DPAT, formulated with or without calcium phosphate, on intra ocular pressure of New Zealand white non-pigmented and Dutch Belted pigmented rabbits of both sex. To understand the role of dopamine D_2 and D_3 receptors in ocular hypotension in pigmented rabbits, the authors employed a dopamine D_2/D_3 receptor antagonist (Raclopride), which did not alter the IOP, but inhibited the ocular hypotension induced by CaP nanoparticles of 7-OH-DPAT, when it was used for pretreatment. In the non-pigmented rabbits, ocular hypotension was more pronounced and sustained when induced by topical administration of CaP nanoparticles of 7-OH-DPAT than nanoparticles without CaP. Further, in the pigmented rabbits, the topical application of 7-OH-DPAT without CaP did not show any change of IOP, but a significant reduction in IOP was noticed with CaP nanoparticles of 7-OH-DPAT. The results concluded that the drug binding to pigments in the anterior segment can significantly limit the therapeutic efficacy. Hence, CaP nanoparticles as drug delivery system can be promising in the treatment of open-angle glaucoma and other anterior segment ocular diseases [37, 39, 40].

Chen *et al.* [36] also employed this CaP nanoparicles system for the delivery of methazolamide, a sulfonamide derivative and carbonic anhydrase inhibitor for the treatment of glaucoma. The methazolamide loaded CaP nanoparticles were reported to have better sustained release than free methazolamide from both *in vitro* and *in vivo* studies. The findings further corroborate promising applications of CaP nanoparticles in glaucoma treatment. Another study involving the use of calcium phosphate nanoparticles was performed by Edelhauser *et al*. [41]. CaP nanoparticles, referred to as nanojackets were loaded with indocyanine green and employed as an imaging tool to obtain the infrared imaging of ocular posterior segment. Hu *et al.* utilized CaP nanoparticles as a carrier tool of DNA for transfection of corneal endothelial cells. Due to high biocompatibility and biodegradable properties of CaP nanoparticles which dissociate into calcium and phosphate ions, gene transfer via CaP nanoparticles was considered a safe alternative to the conventional gene delivery systems [42]. In addition, the calcium phosphate shell facilitated DNA transfer into the nucleus by inhibiting the degradation of DNA by nucleases in the cytoplasm [43].

2.5. Liposomes

Liposomes are vesicular systems with diameters ranging from 50 nm to several microns. Liposomes contain one or more concentric lipid bilayers separated by an aqueous buffer compartment and categorized into 3 primary types: 1. Multi-lamellar vesicles (more than one lipid bilayer) 2. Small uni-lamellar vesicles (10 – 100 nm) and 3. Large unilamellar

vesicles (0.1 – 10.0 μm). Liposomes are capable of encapsulating both hydrophilic (in aqueous compartment) and hydrophobic (in lipid bilayers) drug molecules. Liposomes offer a promising avenue over conventional ophthalmic dosage forms due to protection of drug molecule from degradation by metabolic enzymes on conjunctival and corneal surfaces [44]. Liposomes administered as eye drops were shown to maintain drug therapeutic activity for extended periods of time. In addition, liposomes protect drug molecules from enzymes (proteases and esterase's) in tear fluid. In addition, the liposomal membrane is flexible and supports deformation stress. The property makes them suitable for administering intraocular injections for treating posterior segment ocular diseases [45]. Positively charged liposomes disintegrate completely upon contact with negatively charged mucin membrane [46]. Liposomes have been successfully used in the treatment of eye disorders such as Dry Eye, Keratitis, Endophthalmitis, Uveitis, and Proliferative Vitreoretinopathy [47]. For anterior segment delivery, research is primarily focused on coating the exterior surface of liposomes with bioadhesive and penetration enhancing polymers for improving corneal or conjunctival adhesion and permeation. For posterior segment delivery of liposomes, research is focused on improving intravitreal drug half-life [48]. Schaeffer and Krohn manufactured liposomes loaded with penicillin G and indoxole for topical eye drop therapy. The corneal uptake was highest for positively charged liposomes and lowest for neutral liposomes indicating the electrostatic adsorption between the corneal surface and liposomes [49]. Fitzgerald and colleagues investigated the clearance mechanism of topically administered liposomes in rabbit animal model. The liposomes were prepared by dipalmitoyl phosphatidylcholine or egg lecithin in combination with cholesterol and either dicetyl phosphate or stearylamine. Clearance was monitored by gamma scintigraphy. The study concluded that positively charged liposomes were retained in the pre-corneal area, while large size limited the drainage from the inner canthal region [50]. Mehanna *et al.* described chitosan-based mucoadhesive liposomal formulation of ciprofloxacin HCl employing different concentrations and molecular weights of chitosan. Investigators observed higher ocular permeation of ciprofloxacin HCl and improved antimicrobial activity (gram-positive and gram-negative bacteria) compared to the marketed formulation [51]. Liposomes were also used for the delivery of vectors for genetic transfection, oligonucleotides, and monoclonal antibodies. Bochot *et al.* studied the ocular distribution and clearance of 16-mer oligothymidylate (pdT16) loaded in steric-stabilized liposomes from the vitreous humor following intravitreal injection in rabbit animal model. Competitive hybridization assay was used in testing the integrity of pdT16. Liposomal suspension of [^{33}P]-pdT16 oligonucleotide resulted in higher residual concentrations in vitreous and retina-choroid after intravitreal administration, compared to a simple solution. In addition, liposomes were reported to provide better stability and protection of pdT16 against enzymatic degradation [52]. The US Patent search disclosed 15 patents inventions reporting the successful use of liposomal technology for ophthalmic medications. One such patent is the development of liposomes containing 10–40 mole percent of an aminederivatized lipid component (-NH2 group is separated from a lipid polar head region by a carbon-containing spacer arm). The liposomes with a close packed lipid structure were produced by inclusion of 20–50 mole percent of cholesterol or amine-derivatized cholesterol, and/or phospholipids with predominantly saturated acyl chain moieties. Further, the retention of liposomes on the corneal surface was enhanced by suspending them in an aqueous medium containing a high-viscosity polymer

[53]. Hofland *et al.* [54] reported liposome-based formulations for treating ocular inflammations affecting the cornea, iris, conjunctivita, sclera, and uvea. Liposomes comprising of a neutral lipid (soybean oilbased phospholipid), mucoadhesive agent, a positively charged lipid along with an aqueous phase and therapeutic molecules are disclosed in the patent. The cationic lipid is a member of the group consisting of stearylamine, DC-Cholesterol, dimethyldioctadecylammonium bromide, or 3B-[N′, N′-dimethylaminoethane)-carbamol]. Mucoadhesive compounds include carbopol 934P, polyaxomers, carbomers and plant lectins [54]. The researchers reported that ophthalmic drugs incorporated in these lipid formulations resulted in higher maximum concentration (C_{max}) and longer drug residence time ($T_{½}$) in ocular tissues.

A patent disclosure by Liu *et al.* reported the development of an ophthalmic liposomal formulation for the treatment of neovascularization through selective inhibition of vascular endothelial growth factor (VEGF) [55]. A liposomal system comprising of a phospholipid, charged substance, and/or a membrane-reinforcing substance for delivery to retinal ganglion cells has been patented by Hara and Takeuchi. DNA encapsulated liposomes prepared by dilauroyl-phosphatidylcholine (DLPC) or dioleoylphosphatidylethanolamine (DOPE) and with average particle diameter of ≤600 nm was reported in the patent [56]. Another invention by Kato *et al.* [57] disclosed a liposomal eye drop formulation containing taurine, glucose, asparate and inorganic salts for treating the dry eye or mitigating its symptoms. A patent disclosure by Zeimer *et al.* reported visualizing or treating vasculature using fluorescent dyes/tissue-reactive substances encapsulated within heat-sensitive liposomes designed to release the contents at a pre-determined anatomical locus [58].

In a recent study, Natarajan *et al.* reported the manufacture of liposomes for sustained release of latanoprost, for the treatment of glaucoma. In the study, researchers modified latanoprost-loaded egg-phosphatetidylcholine liposomes using film hydration technique to achieve average particle size of 109 ± 18nm and encapsulation efficiency of 94 ± 5%. *In vitro* release and *in vivo* results demonstrated that liposomes were more efficient in decreasing the intra ocular pressure compared to a daily dose of conventional topical formulation during a 90 day period [59].

In another study, the investigators sought to enhance the bioavailability of ciprofloxacin hydrochloride, an antibacterial used in treating eye infections and corneal ulcers, by formulating the drug in mucoadhesive chitosan-coated liposomes. Entrapment efficiency and drug release of formulations using different molar ratios of L-α-phosphatidylcholine (PC), cholesterol (CH), stearylamine (SA) and dicetyl phosphate (DP) were tested. The research findings concluded that mucoadhesive chitosan-coated liposomes of ciprofloxacin provided prolonged residence time and enhanced bioavailability [60]. In another study, Shafaa *et al.* formulated multilamellar vesicle (MLV) liposomes containing Timolol maleate, an anti-glaucoma drug. *In vivo* studies in rabbit animal model suggested that positively charged MLVs with lower amount of cholesterol reduced the IOP for a prolonged time (~160 hours). Further, the studies indicated that cationic charged and neutral liposomes resulted in superior reduction of intraocular pressure and extended therapeutic effect than anionic charged liposomes [61]. Hathout *et al*. [44] in a study, utilized carbonic anhydrase (acetazolamide) loaded liposomes, manufactured by reversed phase and film hydration

methods, to reduce intra ocular pressure. The studies concluded that increasing the ratio of cholesterol in the formulation and addition of stearylamine resulted in a significant increase in drug entrapment efficiency.

3. NANOTECHNOLOGY – CURRENT & FUTURE DEVELOPMENTS

The first marketed ophthalmic nanoemulsion was approved by FDA in 2002 for the delivery of 0.05% Cyclosporin A (Restasis®) for the treatment of dry eye. Restasis® is a preservative-free anionic nanoemulsion. A recent study by Ammar *et al.* demonstrated successful development of nanoemulsion systems for the delivery of dorzolamide hydrochloride, an anti-glaucoma agent [62]. The research studies concluded that the nanoemulsion formulation exhibited satisfactory physicochemical properties, sustained drug release, higher therapeutic efficacy, fast onset of action compared to the marketed product in solution form (Trusopt®).

Cyclokat® is another novel nanoemulsion formulation of Cyclosporin A, which is currently in the market for the treatment of dry eye disease. The formulation is based on the Novasorb® technology developed by Novagali Pharma, that uses novel cationic nanoemulsion methodology for topical drug delivery [63]. The Novasorb® technology is known to improve bioavailability by interacting with anionic eye surface. Catioprost® is another marketed nanoemulsion formulation of glaucoma drug, latanoprost. The formulation is also based on Novasorb technology and is a preservative-free cationic emulsion [63].

Recently, InSite Vision (USA) developed an innovative drug delivery vehicle, Durasite® (polycarbophil, edentate disodium dihydrate and sodium chloride), as a platform for wide array of ocular drugs. FDA approved a DuraSite formulation containing Besifloxacin to treat bacterial conjunctivitis (pink eye). The technology involves utilizing polycarbophil as a biodegradable matrix for holding drug microparticles for increased drug retention time [64].

CONCLUSION

The review discusses in detail, the development of novel ophthalmic nanoformulation approaches (calcium phosphate (CaP) nanoparticles, nanomicelles, polymeric nanoparticles, soft contact lenses, nanoemulsions, ophthalmic liposomal formulations) and patent inventions reported from promising therapeutic outcomes utilizing the nanoformulation approaches. Recent inventions demonstrated significant improvements in therapeutic outcome with the use of nanoformulation systems for chronic disease states of both anterior and posterior ocular segments. Research studies demonstrated increased drug bioavailability, decreased toxicity and improved therapeutic efficacy with the use of nanoformulations, compared to traditional treatment approaches. From the promising research data reported in patent disclosures, nanoformulation strategy can primarily benefit chronic anterior segment ocular diseases (Glaucoma, Dry Eye, Conjunctivitis, Uveitis, Choroidal Neovascularization) and also posterior segment ocular diseases (Age Related Macular Degeneration). In light of promising inventions, extensive research is in progress to delineate nanoformulation approach applicable for broad ocular disease states.

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Table 1

Summary of recent patented nanoformulations.

Patent No.	Year	Nanoformulation type	Patented nanoformulations	Claimed drugs	Therapeutic use	Reference
US6335022	2002	Nanoemulsion	Nanoemulsion less than 100 nm globule size prepared using oxyethylenated or non-oxyethylenated sorbitan fatty esters	NA*	Ophthalmic	[9]
US6375960	2002	Nanoemulsion	Nanoemulsion prepared using ethoxylated fatty ethers or on ethoxylated fatty esters	NA*	Ophthalmic	[10]
US6656460	2003	Nanoemulsion	Emulsion prepared by mixture of nonpolar phospholipid, a non-polar oil, a non-toxic emulsifying agent, and a cationic lipid	Cyclosporine. Tacrolimus, Sirolimus	Dry eye treatment	[21]
US6998426	2006	Nanoemulsion	Oil-in-water nanoemulsion prepared using nonionic amphiphilic lipids, anionic amphiphilic lipids.	NA*	Ophthalmic	[12]
EP1655021	2008	Nanoemulsion	Oil-in-water type emulsion, prepared using cationic (benzalkonium chloride) non-ionic (tyloxapol), and anionic (phospholipid) with positive charged colloid particles.	NA*	Ophthalmic	[25]
EP1809238	2008	Nanoemulsion	Oil-in-water emulsion (medium chain triglycerides, Tyloxapol, Poloxamer, Vitamin E, Glycerin)	Cyclosporine, sirolimus or tacrolimus	Dry eye treatment	[26]
EP1827373	2008	Nanoemulsion	Cationic ophthalmic oil-in-water type emulsion prepared using Medium Chain Triglycerides, benzalkonium chloride, Lutrol F68™, Tyloxapol)	Latanoprost, travoprost, bimatoprost, tafluprost	Glaucoma	[27]
US20090092665	2009	Nanomicelles	Ophthalmic composition (vitamin E TPGS, octoxynol-40) including mTOR inhibitor	Voclosporin, cyclosporine A, pimecrolimus, tacrolimus, sirolimus, temsirolimus, everolimus	Dry eye syndrome (DES), Sjogren's syndrome, uveitis, conjunctivitis (pink eye)	[32]
US 20090074828A1	2009	Nanoparticle	Stealth nanoparticle using PLGA & PEG to target tissue basement membrane.	Everolimus, zotarolimus, sirolimus, dexamethasone, rapamycin, tacrolimus	Age-Related Macular Degeneration, Choroidal Neovascular iz ation	[33]
US7732404	2010	Nanoemulsion	Pro-nanodispersion formulation prepared using solid fat; tricaprin or ethyl stearate, ethyl lactate, macrogolglycerol hydroxystearate, lecithin at room temperature.	Cyclosporine	Dry eye treatment	[18]
WO2010144194	2010	Nanomicelles	Mixed nanomicellar formulations (vitamin E TPGS, octoxynol-40) of waterinsoluble drugs.	Prednisolone, methylprednisolone, prednisone, triamcinolone, betamethasone, budesonide, and dexamethasone	Posterior ocular segments disease	[29]
US20110008421	2011	Liposome	liposome to target posterior segment of the eye and prepared by phospholipid, a charged substance and a membranereinforcing substance	6-cumarin	Posterior segment	[56]

Patent No.	Year	Nanoformulation type	Patented nanoformulations	Claimed drugs	Therapeutic use	Reference
US8298568	2012	Nanoemulsion	Oil-in-water type emulsion (cetalkonium chloride, tyloxapol and poloxamer) with average particle size of about 300 nm and positive zeta potential.	Sirolimus	Uveitis	[22]
US8097270	2012	Nanoparticles	Inorganic Nanoparticles (hydrous clays, and silica) composed in contact lenses containing surface-active biocide.	Polyquaternium-1	Antimicrobial, glaucoma	[35]
US8273366	2012	Nanoemulsion via contact lens	Drug encapsulated polymeric nanoparticles dispersed in contact lens (poly 2hydroxyethylmethacrylate).	Prednisilone acetate, gentamycin, cephalosporin, lidocaine, timolol, ciprofloxacin, cyclosporin A, or pilocarpine	Anti- inflammatory, Antiinfective, Glaucoma	[28]
US8153156 B2	2012	Nanoparticles	Nanocomposite by reversible hydrogel embedded nanoparticles.	NA*	Substitute of vitreous humor	[34]
US8414904B2	2013	Nanoemulsion	Oil-in-water emulsion with neutral zeta potential.	Prostaglandin	Glaucoma	[15]
WO2004050065A1	2004	Nanoparticle	Calcium Phosphate core particles	NA*	NA General ocular drugs.	[37]

*NA: No drug is claimed.