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Controlled Ocular Drug Delivery with Nanomicelles

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Abstract

Many vision threatening ocular diseases such as age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, and proliferative vitreoretinopathy may result in blindness. Ocular drug delivery specifically to the intraocular tissues remains a challenging task due to the presence of various physiological barriers. Nonetheless, recent advancements in the field of nanomicelle based novel drug delivery system could fulfil these unmet needs. Nanomicelles consists of amphiphilic molecules that self-assemble in aqueous media to form organized supramolecular structures. Micelles can be prepared in various sizes (10 to 1000nm) and shapes depending on the molecular weights of the core and corona forming blocks. Nanomicelles have been an attractive carriers for their potential to solubilize hydrophobic molecules in aqueous solution. In addition, small size in nanometer range and highly modifiable surface properties have been reported to be advantageous in ocular drug delivery. In the present review various factors influencing rationale design of nanomicelles formulation and disposition are discussed along with case studies. Despite the progress in the field, influence of various properties of nanomicelles such as size, shape, surface charge, rigidity of structure on ocular disposition need to be studied in further details to develop an efficient nanocarrier system.

Introduction

There are many vision threatening ocular diseases such as age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, and proliferative vitreoretinopathy that may result in blindness. Chronic nature of these diseases requires frequent drug administrations to

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maintain visual acuity and halt disease progression. For example, intravitreal (IVT) administration of anti-VEGF (vascular endothelial growth factor (VEGF)) therapy slows progression of AMD. From drug delivery perspective, the eye can be divided in three segments, precorneal area, anterior segment and posterior segment. Clinically therapeutic agents are administered by topical, systemic and, recently, IVT routes. The anatomy of the eye and various routes of administrations are shown in the Fig. 1.

Topical route is most patient compliant and suitable for diseases affecting anterior segment. The precorneal factors such as tear turnover and drainage, dilution by tear flow, reflex blinking and lacrimation shortens residence time for topically instilled conventional dosage forms. Precorneal factors also lower the concentration gradient which is a driving force for passive absorption/permeation of drugs across the cornea and conjunctiva resulting in poor ocular bioavailability (<5%)¹. Therefore, repeated administrations are indicated to maintain therapeutic drug levels. The tear film is composed of three layers: the innermost mucin film, the middle aqueous layer, and the outer oily layer that retards water evaporation from tear film. Complex structure of the tear film can retard the absorption of drugs into the cornea and sclera². When the drop volume is greater than 30 μL (volume that can be accommodated in the cul-de-sac without spillage³), nasolacrimal drainage and drainage owing to gravity are major pathways for drug loss.

The cornea consists of five different layers, i.e., epithelium, Bowman membrane, stroma, Descemet's membrane, and endothelium⁴. The corneal epithelium possesses tight intercellular junctions that prevent uptake and paracellular permeation of polar molecules. The corneal stroma is hydrophilic, which hinders rapid movement of hydrophobic molecules. However, the epithelium is lipophilic and offers resistance to passage of hydrophilic molecules. For topically instilled drugs, the corneal route is considered as a major pathway for absorption into intraocular tissues. Recently, attention has been shifted to the trans-scleral route due to presence of physiological barriers, which may provide higher drug levels in intraocular tissues following topical instillation. The sclera is not a major barrier for the transport of small molecules, but it impedes permeation of macromolecules. Similarly, the conjunctiva is relatively porous allowing ready passage of small molecules. However, absorption of drugs in the lymph and blood circulations in the conjunctiva could eliminate a significant amount of therapeutics in the systemic circulation^{5, 6}.

The blood-ocular barriers (BOB) consists of the blood-aqueous barrier (BAB) and blood-retinal barrier (BRB) which acts as major barriers to drug entry into the retina after systemic and periocular administrations. BAB restricts the access of xenobiotics to the anterior segment. BAB is not considered a complete barrier because of the fenestrated capillaries on the ciliary processes. These capillaries are highly permeable and allow ready passage of small molecules^{7, 8}. Therefore, small molecules present in the aqueous humor, can easily enter the iridial circulation and be eliminated from the anterior chamber⁹. BRB is further divided into inner and outer BRB. It is present in the posterior segment and prevents the entry of drug molecules from blood circulation into the retina. The outer BRB is formed by the monolayer of retinal-pigmented epithelium (RPE) having intercellular tight junctions¹⁰. The inner BRB is composed of tight junctions between the endothelial cells of the retinal blood vessels. Anatomically, Inner BRB is quite similar to the blood-brain barrier (BBB). In

comparison to BBB, the inner BRB has a greater density of tight junctions and pericytes¹¹. The glial cells and the tight junctions of the endothelial cells together limit the entry of xenobiotics into the retina¹².

Drug delivery to the intraocular tissues continues to be a challenging task. Nonetheless, recent advancements in the field of nanotechnology based novel delivery systems are being designed to fulfil these unmet needs. In the present review, we have discussed applicability of nanomicelles in controlled ocular drug delivery (ODD). Factors influencing the ODD are also discussed along with published case studies for rational design of nanomicellar carries.

NANOMICELLES IN OCULAR DRUG DELIVERY

Micelles consists of amphiphilic molecules that, generally, self-assemble in aqueous media to form organized supramolecular structures. Micelles are formed in various size (10nm to 1000nm) and shapes (spherical, cylindrical and star-shaped, etc.) depending on the molecular weights of the core and corona forming blocks. The self-assembly take place above certain concentration, referred to as critical micelle concentration (CMC). A schematic representation of micelle formation with amphiphilic polymers or surfactants is shown in the Fig. 2. The force driving the self-assembly and maintenance of supramolecular assembly is hydrophobic interactions of core forming blocks, for typical micellar structures. The corona-forming block is water soluble that renders micelles soluble in aqueous phase. Taking the advantage of hydrophobic core, the nanocarriers can be utilized to enhance the water solubility of hydrophobic molecules.

Nanomicelles investigated for ODD thus far can be divided into three broad categories i.e., polymeric, surfactant and polyionic complex (PIC) micelles. The typical surfactant micelles are characterized by higher CMC where a dynamic equilibrium exists between micelle aggregates and unimers in the solution. Micellar aggregates formed by surfactants are weak and susceptible to physical instability upon dilution. In contrast, polymeric nanomicelles exhibit lower CMC and better stability against dilutions. Hydrophobic drugs are encapsulated in the nanomicelle core by hydrophobic interaction. Other hydrophobic interactions, forces such as van der Waals' interactions and hydrogen bonding may also contribute to the encapsulation in micelle core. The polymer forming PIC micelles are water soluble with a charged core-forming block. Electrostatic interaction between core-forming block and oppositely charged active pharmaceutical ingredient (API) acts as the driving force for micelle formation and stabilization of supramolecular assembly. Selection of the type of nanomicelle carrier is dependent on the physicochemical properties of drug molecule, drug:polymer or drug:surfactant interactions, site of action, rate of drug release, biocompatibility and physical stability. For example, PIC micelles would be best suited for poly-negatively charged bio-therapeutics such as antisense oligonucleotides and plasmid DNA. A summary of micellar formulation investigated for ODD is represented in table 1.

Polymeric Nanomicelles

Polymeric nanomicelles are formed by amphiphilic polymers with distinct hydrophobic and hydrophilic segments. The polymer self-assemble to form micelles in aqueous solution, wherein water insoluble segment forms the core and hydrophilic segment forms the corona.

In some cases, the self-assembly is not spontaneous and micelle formation is assisted by additional means, such as temperature¹³. The self-assembly occurs above the CMC. The hydrophilic segments forming corona aid the solubilization of entire supramolecular structure. Polymeric micelles are characterized by their low CMC in addition to excellent kinetic and thermodynamic stability in solution.

Ideally the polymers utilized to prepared nanomicelles should be biodegradable and/or biocompatible. The most widely studied core-forming polymers are poly(lactide), poly(propylene oxide) (PPO), poly(glycolide), poly(lactide-co-glycolide), and poly(ϵ -caprolactone) (PCL). Poly(ethelene glycol) (PEG) is the most frequently utilized hydrophilic segment due to its excellent water solubility and biocompatibility. Structures of various micelle forming amphiphilic polymers and surfactants are shown in the Fig. 3. Biodegradation of polymer ensures elimination of inactive polymer from ocular tissues. However, the degradation products should not be toxic or inflammatory to the sensitive ocular tissues particularly the neural retina.

There are several important attributes that must be deliberated to rationally design a nanomicellar formulation. Some of the important factors are site of action, polymer composition, drug loading, release rate, nanomicelle-tissue interaction, size and surface charge. Hydrophobic drug is encapsulated in the micelle core during or after micelle formation depending on the preparation method. The process involves hydrophobic interactions and/or hydrogen bond formation between drug and polymer. The most commonly used methods of micelles formation are direct dissolution, solvent evaporation, film hydration and dialysis method¹⁴. Encapsulation efficiency in the micelle core depends on the method used to prepare the micelles and extent of drug-polymer interactions. Generally, methods like solvent evaporation and film hydration result in higher encapsulation efficiency than direct dissolution and dialysis methods^{14, 15}. For example, aqueous solubilities of biphenyl dimethyl dicarboxylate in mPEG₂₀₀₀-PLA₁₀₀₀ carrier system with film hydration method was 13.2 mg/ml compared to 2 mg/ml with dialysis method¹⁶. Direct dissolution is the simplest method of preparation and may be easy to scale up. Depending on the type of polymer and drug-polymer interaction, this method may also be modified to eliminate the use of organic solvents like acetone or DMF that must be removed before clinical use. For example, honokiol was encapsulated in nanomicelles using direct dissolution methods without using organic solvents. Triblock co-polymer PCL-PEG-PCL was dissolved in water to form micelles by heating at 50°C followed by entrapment of honokiol¹⁷. Film hydration method can also be an alternative to direct dissolution method. Exposure of polymer-drug film for extended duration under vacuum could completely remove volatile organic solvents³.

Stability of nanomicelles is very important for efficacious ODD. As mentioned earlier, polymeric nanomicelles are kinetically and thermodynamically more stable compared to low molecular weight surfactant micelles. The rate of dissociation of unimers from polymeric micelles is slower making the micelles kinetically stable¹⁸. Thermodynamic stability is achieved by interactions of core-forming blocks as well as the ability of hydrophilic block to solubilize the supramolecular structure. In addition, stability of nanomicelles may also improve upon incorporation of hydrophobic drug molecules⁴. Nonetheless, the stability of

nanomicelles in various ocular tissues should be examined since the nanomicelles are exposed to various cellular environment. For instance, physical stability of topically instilled formulation should be evaluated in precorneal tear fluid for developing successful ODD system.

The mechanism of drug release from nanomicelles is dependent on the nature and strength of interactions between core-forming polymer and drug molecules, micelle stability in ocular tissues, polymer degradation and rate of diffusion of drug molecules from micelle core. Drug release should be tailored keeping the site of action in mind. For example, if the site of action is at precorneal area such as conjunctiva or cornea, drug release must take place in the precorneal space and the released drug may be absorbed, following topical administration. Nevertheless, nanomicelles must provide good precorneal retention time to avoid loss of formulation via precorneal clearance mechanisms. In a more productive absorption scenario, topically administered nanomicelles may be absorbed followed by release in the target tissue. Stability of nanomicelles in the precorneal environment and cell-micelle interactions may determine length of precorneal residence time. Volume of lachrymal fluid is 7 μL and normal tear turnover rate is 0.66 $\mu\text{L}/\text{min}$ ¹⁹. However, reflux tearing may remove a significant amount of drug which can be avoided by maintaining appropriate size ($<5\text{ }\mu\text{m}$), isotonicity and osmolarity of nanomicelle solution. On the other hand, vitreous humor is relatively stagnant compartment and the release rate could be dependent on physical stability, polymer degradation rate and drug-polymer interaction. However, no nanomicelle systems has been able to provide sustained release for more than a few days. Thus nanomicelles delivery via IVT route would require frequent administrations. Frequent IVT injections are not patient compliant and associated with various side effects such as endophthalmitis, retinal detachment and retinal haemorrhage. Hence, nanomicelle systems should be avoided for IVT administration.

Size and surface charge of the nanomicelles are critical aspects of designing an ODD system. For polymeric micelles, size can be easily controlled by the molecular weight of the polymer and drug loading. Ocular disposition of nanomicelles following topical instillation may be influenced by the size of nanomicelles²⁰. Transport of nanocarriers such as nanoparticles have been reported to be dependent on the size i.e., smaller the size higher is the permeability of nanocarrier²⁰. Paracellular transport of nanomicelles across the conjunctiva and the sclera may result in higher drug levels in the intraocular tissues following topical administration. In addition, size of nanomicelle may influence cellular entry processes such as endocytosis. It may be the primary mechanism for drug uptake in corneal and conjunctival cells. Nanomicelles of pilocarpine were developed with amphiphilic pluronic (F127, Mw 12,600) polymer²¹. Pluronic polymers are triblock copolymers having hydrophilic PEG flanking the hydrophobic PPO. Above CMC, F127 forms nanomicelles of size $\sim 17\text{nm}$ and $\sim 23\text{nm}$ in DDI water and PBS buffer, respectively. Thus the size of nanomicelle increased in the presence of electrolytes. In addition, incorporation of hydrophobic agents into micelle core also increased the micelle size. Nanomicelle size of $\sim 30\text{nm}$ was observed for pilocarpine base loaded nanomicelles in PBS, pH 7.4. When these nanomicelles were examined for miotic response in female albino rabbits, a higher effect and longer duration of mitotic response was observed for pilocarpine base-loaded pluronic

micelles. Such augmentation in mitotic response may be due to productive absorption of drug-loaded nanomicelles or released hydrophobic pilocarpine base form.

In order to improve stability of pluronic micelles, poloxamine class of polymers were investigated. Poloxamines are branched form of poloxamers and have been shown to possess better stability. Unlike pluronic, poloxamine polymers are also pH responsive apart from being temperature responsive. Ribeiro A et al., have developed poloxamine polymers-based single and mixed micelles to deliver ethoxzolamide via topical administration²². Various tetronic co-polymers with varying HLB values were studied for ethoxzolamide solubilization, kinetic stability and drug release. Low molecular weight T904 based single micelles exhibited unimodal distribution (~22nm) unlike poloxamine with high molecular weight (T1104 and T1307; ~52-65nm). Most mixed micelles exhibited a multimodal distribution except for T904:T1107 and T904:1307 micelles at the ratio of 75:25. Multimodal size distributions were also observed for all the mixed-micelles where high molecular weight poloxamines were added at higher ratios. However, when low molecular weight T904 was combined with high molecular weight T1107 and T1307 at 75:25 ratio, the resulting mixed-micelles exhibited micelle sizes close to that of T904 based micelles with unimodal distribution. Also the mixed micelles of T904:T1107 and T904:1307 (75:25 ratio) displayed higher cloud points (CP) (76°C and 71°C) compared to T904 alone (65°C) suggesting improved stability of mixed micelles. A further rise in CP was observed when fraction of hydrophobic poloxamine was elevated. However, along with improved stability, a multimodal size distribution and higher average micelle size was observed for mixed-micelles. Ethoxzolamide release lasted over 1-5 days for all nanomicelle systems and was found to be slower for poloxamine with more hydrophobic core. Release rate was intermediate when hydrophilic T904 was added to T1107 and T1307. Thus rate of release could be tailored as per the drug delivery requirement using these mixed micelle systems.

Surface charge could influence the cell-micelle interactions and thus play a critical role in determining ocular disposition of nanomicelles from precorneal space. Cell surface and mucin layer over the cornea are negatively charged. Hence, developing a nanomicelle formulation having positive surface charge may prolong the precorneal residence time and enhance transcorneal permeability by promoting favourable interaction between the nanomicelle and ocular surface. In order to increase the precorneal residence time and thus bioavailability, several attempts have been made to improve cell-micelle interaction. Two of such strategies include modifying nanomicelle surface with cationic polymer such as chitosan and modifying core-forming block with lipid chains.

Chitosan is a cationic polysaccharide with bioadhesive property. Chitosan polymer is hydrophilic in nature. Being a cationic molecule, it could facilitate the interaction of nanomicelles with negatively charged cell surface enhancing precorneal retention and ocular bioavailability. Pepic et al., incorporated chitosan in dexamethasone (DEX)-loaded poloxamer (F127, Mw 12,600 Da) micelles to improve corneal absorption²³. Chitosan was incorporated in nanomicelles at 0.005, 0.01 and 0.015% w/v. Low concentrations of chitosan did not exhibit significant effect on nanomicelle size, drug loading or drug release (Fig. 4). However, it significantly raised zeta potential by 10-17 fold. At 0.01 and 0.015%, chitosan also enhanced the permeability of DEX across caco-2 monolayer. Ocular bioavailability was

measured by determining change in IOP. A 1.7-fold increase in AUC was observed with F127 nanomicelles compared to commercial DEX eye drops (0.1% w/v) (Table 2). Enhancement in bioavailability could be attributed to smaller size (~22 nm). In addition, a 2.4 fold increase in AUC was observed with chitosan-modified F127 nanomicelles. This enhancement could be due to favourable electrostatic interaction of nanomicelles with corneal surface.

In another study, chitosan/F127 micelles were prepared for metipranolol at higher chitosan concentrations (0.3-0.8%). At these concentrations chitosan improved metipranolol loading and raised micelle size along with zeta potential²⁴. However, a significant burst release was observed upon incorporation of chitosan at high concentration. Metipranolol release from F127 micelles was ~28 % at first hour. However, first hour drug release from chitosan/F127 micelle was 59%, 69%, and 88% with 0.3%, 0.5% and 0.8% of chitosan content, respectively. Despite immediate release, 0.5% chitosan/F127 micelles were able to increase AUC of metipranolol by 1.67 fold relative to commercial eye drops. In contrary, metipranolol-loaded F127 micelle exhibited similar AUC as of commercially available eye drops. Hence, increment in the AUC observed for chitosan/F127 micelles could be attributed to bioadhesive property imparted by chitosan. No significant difference in AUC was observed when F127 was compared against commercially available eye drops of metipranolol. It could be attributed to the lack of bioadhesion leading to elimination of micelles from precorneal area.

In a yet another approach to improve the cell-micelle interactions and bioavailability, lipid modified block co-polymer poly(ethylene glycol)-poly(hexyl-lactide) (mPEG-hexPLA) was developed to deliver hydrophobic molecule cyclosporine A (CyA)²⁵. CyA is an immunosuppressant, which acts by inhibiting interleukin-2 release²⁶. It is prescribed for various ophthalmic conditions such as corneal graft rejection, autoimmune uveitis and dry eye syndrome. CyA is a hydrophobic molecule (log P 2.92) and has poor aqueous solubility (12 µg/mL)²⁷. Tommaso, C et al., have developed and characterized CyA-loaded nanomicelles for the treatment of dry eye syndrome and prevention of corneal graft rejection following topical administration. The di-block co-polymer mPEG-hexPLA was utilized for the preparation of CyA-loaded micelles. A stable nanomicelle solution resulted in CyA concentration as high as 1.5 mg/ml and nanomicelles size of 51.4 ± 0.4 nm with unimodal distribution. Nanomicelles were found to be nontoxic and biocompatible in cytotoxicity assay and ocular tolerability (rabbit model) experiments²⁸. mPEG-hexPLA nanomicelles were tested in healthy rat model as well as rat model of penetrating keratoplasty²⁹. Nanoformulation was able to overcome the precorneal and corneal barriers. A significantly higher level of CyA was observed in cornea (6470 ± 1730 ng/g_{tissue}) with nanocarrier system, which was 11-fold higher than oil-based formulation of CyA. Higher corneal levels resulted in better graft tolerability in rat corneal keratoplasty model. Further *in vitro* experiments in corneal primary cells suggested that mPEG-hexPLA based nanocarriers are capable of interacting and entering the corneal cells. Thus these nanomicelles may be stable in the precorneal fluid and can permeate into the cornea. Nanomicelles may release CyA in the cornea resulting in significantly higher levels of CyA in the healthy rat model³⁰.

The eye is a specialized and isolated organ where most nutrients are supplied via specialized transporter mechanisms. Sterility and biocompatibility are very important aspects that must be studied thoroughly before selecting the type of polymers and excipients. Polymeric micelles have been recently investigated for their potential as ODD. One advantage with nanomicellar carrier is that it can be easily filter sterilized to lower the endotoxin/microbial burden. There are only a few publications available discussing safety and biocompatibility of polymeric carriers in various ocular tissues. Lu Xu et al., have studied biocompatibility of mPEG-PCL micelle system (Mn 4000) in rabbit eyes³¹. No significant change in IOP was observed following single intracameral injection of mPEGPCL nanomicelles (200 µl of 200 mg/mL solution, micelle size ~50 nm) over the period of 15 days. Changes in microstructure of cornea and retina were also studied following intracameral and IVT injections respectively, by H&E staining. Histopathological analysis showed no change in microstructure of cornea and retina after third and thirtieth day of nanomicelle administration. Similarly, polylactide and lipid based polymer mPEG-HexPLA was also found to be biocompatible with ocular tissues³². These studies suggest biocompatible nature of above mentioned polymers following a short term exposure. It is worth noting that most ocular ailments are chronic and therefore a long term toxicity study may shed light on safety and biocompatibility.

Surfactant Nanomicelles

Amphiphilic molecules having a hydrophilic head and hydrophobic tail are commonly referred to as surfactants. Hydrophilic head of surfactant molecules can be dipolar/zwitterionic, charged or anionic/cationic, or neutral/non-ionic. Commonly used surfactants for nanomicellar formulation are: sodium dodecyl sulfate (SDS, anionic surfactant), dodecyltrimethylammonium bromide (DTAB, cationic surfactant), ethylene oxide (N-dodecyl; tetra, C12E4), Vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate), octoxynol-40 (non-ionic surfactants) and dioctanoyl phosphatidyl choline (zwitterionic surfactants). A hydrophobic tail commonly comprises of a long chain hydrocarbon and rarely includes a halogenated/oxygenated hydrocarbon/siloxane chain.

Micelles are formed when surfactants are dissolved in water at concentration above CMC. A balance between intermolecular forces such as Van der Waals interactions, hydrogen bonding, hydrophobic, steric and electrostatic interactions are vital for nanomicellar formulation. Shape of nanomicelles i.e. spherical, cylindrical or planar/discs/bilayers depends on the non-covalent aggregation of surfactant monomers. Alteration in the chemical structure of surfactant and conditions such as surfactant concentration, pH, temperature, ionic strength may determine the shape and size of nanomicelles. Transformation of nanomicelles can take place from one dimension into cylindrical micelles or two-dimension into bilayers/discoidal nanomicelles, which can be controlled by surfactant heads. Such transformation results from reduced forces of repulsion between the charged head groups.

Nanomicellar formulation for the topical delivery of small as well as macro-molecules has been exploited by several investigators. Surfactant nanomicellar formulation has been utilized for improving the diffusion of topically delivered drugs through cornea thus improving ocular bioavailability. Luschmann C et al., developed a nanomicelle solution of

cyclosporine A (CyA) containing non-ionic surfactants (Sympatens AS)³³. The average size of formulated nanomicelles ranges between 9.7 and 10.1 nm. Nanomicelle solution showed no signs of ocular irritation. It may be considered as a seamless drug delivery system for administration of CyA to the anterior segment. It also exhibited high levels of CyA in cornea. The nanomicelle solution exhibited high levels of CyA (826 ± 163 ng/g_{cornea}), which exceeded the tissue levels reported for cationic emulsion of CyA (750 ng/g_{cornea}) and Restasis® (350 ng/g_{cornea}). Therefore, nanomicelles of CyA promises an efficient method of treatment for inflammatory corneal diseases and may improve the patient compliance by reducing the number of instillations per day.

Vadlapudi et al., developed a clear, aqueous nanomicellar formulation of biotinylated lipid prodrug for the treatment of corneal herpetic keratitis³⁴. Non-ionic surfactants – vitamin E TPGS and octxynol-40 were selected to formulate micellar formulation of biotin-12Hydroxystearic acid-acyclovir (B-12HS-ACV). TEM analysis suggested that nanomicelles were spherical, homogenous and devoid of aggregates. The average size of formulated nanomicelles was 10.78 nm. No significant burst effect we reported for the release of B-12HS-ACV from nanomicellar formulation. A sustained release of B-12HS-ACV from its nanomicellar formulation was observed for a period of 4 days as compared to 100% release of B-12HS-ACV in ~6 h from its ethanolic solution.

Kuwano M et al., compared CyA pharmacokinetics and distribution in rabbit ocular tissues resulting from topical application as an oil-based medium, o/w emulsion, and aqueous clear solution containing a surfactant³⁵. Polyoxyethylene 20 sorbitan monooleate (Tween 80), polyoxyl 40 stearate (MYS-40) and polyoxyl 60 hydrogenated castor oil (HCO-60) were utilized for the preparation of CyA nanomicelle formulation. Higher solubility of CyA was obtained in MYS-40 formulation relative to other formulations. Increase in the solubility of CyA was reported with increase in the weight percentage of MYS-40 at a constant temperature. The *in vivo* ocular tissue distribution and pharmacokinetic studies were carried out with 0.1% CyA nanomicelles. Blurred vision and ocular irritation were reported as side effects associated with oil based CyA formulation and are thus considered undesirable for ocular administration. Improved ocular drug accumulation was reported with single topical drop of CyA-MYS-40 nanomicelle aqueous formulation. Higher CyA Levels (ng-eq/g) were reported in the posterior ocular tissues after single instillation (50 µL) post 24 h i.e. 1.54 ± 0.95 (Aqueous-CyA) $> 0.92 \pm 0.57$ (Oil-CyA) $> 0.36 \pm 0.08$ (Emulsion-CyA) (Table 3). In all the ocular tissues concentration of CyA was higher with MYS-40 aqueous formulation compared to oil- and emulsion-based formulations. There was increase in AUC with aqueous-CyA formulation in corneal stroma–endothelium, bulbar conjunctiva and lacrimal gland as compared to oil- and emulsion-based formulation. CyA exhibited the following intraocular penetration order: aqueous-CyA $>$ emulsion-CyA $>$ Oil-CyA formulation. Chemical properties of vehicle may be responsible for the different intraocular drug penetration. Hydrophobicity of the vehicle may govern the release of CyA from the formulation. Drug partitioning of CyA was low in case of oil-based formulation because of the hydrophobic nature of vehicle/carrier or in this case “oil”, which resulted in low amount of drug availability for permeation into deeper ocular tissues. However, the hydrophobic drug dissolves easily in presence of non-ionic surfactants due to formation of micelles.

These micelles possess large surface area relative to the surface area of emulsion droplets and are of size >200nm which results in higher drug release. Topical aqueous surfactant micelles also help in avoiding the side effects associated with emulsion-and oil-based formulations. Topical surfactant aqueous nanomicellar formulation can also be employed to non-invasively deliver therapeutic agents to posterior ocular tissues.

Mitra et al. reported application of nanomicellar formulation for posterior segment delivery via topical administration³⁶. Vitamin E TPGS and octoxynol-40 of different hydrophilic lipophilic balance (HLB) values were used to prepare aqueous mixed nanomicellar formulation of voclosporin in order to carry out initial studies. Rapamycin and DEX were also encapsulated in an aqueous nanomicellar formulation. Size of nanomicelles encapsulating voclosporin, rapamycin and DEX were in the range of 10-25 nm. Voclosporin aqueous mixed nanomicellar formulation (0.2%) efficacy was compared with Optimmune® (CyA ophthalmic ointment) in canine keratoconjunctivitis sicca model utilizing Schirmer tear test (tear production in an eye) and corneal observation as end points. The control values were found way below the threshold value (>15mm/min) whereas nanomicellar formulation maintains the value well above the threshold. No side effects were noticed with voclosporin nanomicellar formulation administered twice daily indicating its safety in animal model. Tolerability studies of nanomicellar formulations (0.02 and 0.2%) against Restasis® was investigated in New Zealand White (NZW) rabbits. Ocular irritation was reported highest in Restasis® compared to voclosporin nanomicelle formulation. These results confirmed that voclosporin aqueous nanomicelle formulation is well tolerated and induce significantly less ocular irritation as compared to Restasis®. Voclosporin (0.2%) nanomicelle formulation showed no dose dependent side effect on particular function and histopathologic ocular indices in 2 and 13 week studies carried out in NZW rabbits and beagle dogs. No toxicity with minimal systemic exposure and accumulation were observed with nanomicelle formulation. Anterior and posterior tissues were analysed for voclosporin levels following single and once daily drop instillation of nanomicellar formulation in NZW rabbits. High drug concentrations were reported in the posterior ocular tissues in relative to minimal and/or non-detectable drug levels in aqueous humor, lens and vitreous humor (Table 4). Adverse effects such as increased intraocular pressure or cataract formation can be avoided with nanomicelle formulation due to the minimal drug levels in aqueous humor, lens and vitreous humor. Mixed nanomicellar aqueous formulations can be utilized to deliver therapeutic agents to the posterior ocular tissues via topical instillation.

Recently, DEX and rapamycin topical nanomicellar formulations were utilized for posterior ocular tissues delivery via non-invasive route³⁷. Encapsulation of DEX and rapamycin in nanomicellar formulation resulted in improved solubility of DEX and rapamycin by 6.7 and 1000 times, respectively. Ocular tissue distribution studies revealed that 50 ng/g and 370 ng/g of DEX and rapamycin, respectively were detected in retina-choroid whereas minimal or no drug levels were detected in aqueous ocular chamber suggesting a non-corneal route of drug absorption to the posterior segment.

Chopra P et al., investigated the feasibility of mixed micellar system prepared with sodium taurocholate (surfactant) alone or with egg lecithin (lipid) as carrier system for sustained delivery of DEX in transscleral iontophoresis³⁸. With higher total lipid concentration, the

solubilization capacity of micellar system also increased. In comparison to DEX solution, DEX release from the sclera was significantly prolonged with the micellar carrier systems after passive and iontophoretic delivery. After 2 h of cathodal iontophoretic delivery of the micellar carrier systems, less than 20% of DEX was released from the sclera in comparison to ~50% of DEX released from the control DEX solution without micellar carriers. Micellar carrier systems not only enhance aqueous solubility of DEX but also provided sustained release of DEX from the carrier system. Thus it can be regarded as a suitable transscleral drug delivery system for poorly water soluble drugs. Improved sclera loading and delayed drug release from the sclera were regarded as two major factors for micellar carrier-sustained DEX delivery. Micellar carrier system enhanced the aqueous solubility of DEX which in turn enhances the delivery of DEX into the sclera following iontophoresis. This process ultimately results in a greater amount of DEX released from the sclera as compared to control. Interaction of carrier system with sclera i.e. binding of micelles with sclera could be postulated as a reason for delayed DEX release from the sclera (Fig. 5).

Civiale C et al., utilized polyhydroxyethylaspartamide (PHEA), bearing side chains of polyethylene glycol (PEG) and/or hexadecylamine (C16) (PHEA-PEG, PHEA-PEG-C16 and PHEA-C16) as potential colloidal drug carriers for ocular drug delivery³⁹. The investigators formulated netilmicin sulphate, DEX alcohol and dexamethasone phosphate-loaded PHEA-C16 and PHEA-PEG-C16 micelle carriers. Drug-loaded PHEA-C16 and PHEA-PEG-C16 micelles exhibits higher permeation across ocular epithelia than drug in solutions or suspensions. PHEA-PEG-C16 was found to be the best permeability enhancer compared to PHEA-C16 based on *in vitro* experiment. In an *in vivo* biodistribution study in rabbits, a 40% increment in AUC_{aq humor} was observed with DEX loaded PHEAPEG-C16 micelles (9494 ng/mL*min) compared to control (5976 ng/mL*min) following topical administration (Fig. 6).

PIC nanomicelles

Polyion complex (PIC) micelles have been widely investigated as nanocarrier system for gene and antisense oligonucleotide delivery^{40, 41}. In particular, PIC micelles have been explored extensively for the delivery of ionic hydrophilic therapeutics⁴²⁻⁴⁵. This special class of micelles are formed by electrostatic interactions between polyion copolymers (comprised of neutral segment and ionic segment) and oppositely charged ionic species. The block copolymer is water soluble with very narrow polydispersity⁴⁴. Neutral segment of block copolymer is usually PEG whereas the ionic segment is neutralized by oppositely charged species to form hydrophobic core⁴⁶. For drug delivery applications, ionic drug serves as an oppositely charged species. In aqueous medium, non-ionic hydrophilic segment (PEG) stabilizes hydrophobic polyion-drug complex and forms self-assembled corona-core structure (PIC micelles). Formation of PIC micelles were first reported by Harada et al. where investigators have utilized poly(ethylene glycol)-poly(L-lysine) (PEG-P(Lys)) and poly(ethylene glycol)-poly(α,β -aspartic acid) (PEG-P(Asp)) block copolymer for the construction of micelles⁴⁷. PIC micelles have been extensively studied for gene delivery, where block copolymer is comprised of neutral hydrophilic segment (PEG) and polycationic segment. A schematic representation of PIC micelle formation is illustrated in Fig. 7. In this

system, charges of DNA are neutralized by polycations which forms hydrophobic core of the PIC micelles^{48, 49}.

Various research groups have explored applicability of PIC micelles for ocular applications. Recently, Ideta et al., have formulated PIC micelles encapsulating model agent and investigated their applicability for the treatment of choroidal neovascularization (CNV)⁵⁰. PIC micelles comprised of PEG-P(Asp) and model drug (FITC-P(Lys)) exhibited ~50 nm micelle size with very narrow polydispersity index of 0.046. Investigators have studied tissue distribution of PIC micelle following intravenous injection in rat CNV model. PIC micelle treated group depicted accumulation of FITCP(Lys) as early as 1 h and the level peaked at 4 h with a residence time of 168 h. Accumulation of PIC micelles in CNV lesions may be attributed to enhanced permeation and retention (EPR) effect since neovascular vessels in CNV are highly permeable and poorly developed. In addition, PIC micelles exhibited excellent stability and improved blood circulation which may also have contributed to high localization of FITC-P(Lys) in CNV lesions.

Photodynamic therapy (PDT) is efficient treatment modality for corneal neovascularization. Photosensitizers are given intravenously, which accumulate in newly formed vasculature. Photosensitizers then release reactive oxygen species upon activation by mild laser application. However, due to the non-specificity many incidences of skin toxicity are reported. In order to improve targetability of dendrimer zinc porphyrin (DP, photosensitizer), investigators have prepared and evaluated PIC micelles consisting of PEG-P(Lys) block copolymer⁵¹. Taking advantage of EPR effect, PIC micelles tend to accumulate in neovascular tissue following intravenous delivery. Results clearly suggested significantly higher fluorescence in DP-micelle treated corneas relative to free DP treated animals suggesting improved targetability of DP upon encapsulation in PIC micelles.

The same research group has investigated applicability of DP-micelles for the treatment of CNV in wet-AMD⁵². Nanomicelles exhibited significantly higher accumulation of DP in CNV lesions relative to free DP. Moreover, the study demonstrated more than 80% of CNV occlusion at 4 h post PDT treatment. Moreover, DP-micelle treatment group exhibited no signs of photo-induced erethism after 2 weeks, following irradiation, suggesting excellent safety profile of DP-micelle formulation. Due to the targetability with reduced side effects, this new technology offers tremendous promise for ocular delivery of ionic macromolecules.

Conclusion

Nanomicelles have received considerable attention as ODD carriers recently. However, there are a number of unanswered questions related to clinical applicability and success of this formulation as ODD carrier. These carriers definitely hold potential to improve ocular bioavailability and patient compliance. Nanomicelles may be developed for intraocular and surface ocular diseases to deliver API by the most patient compliant topical route. Investigations with various nanomicelles in the last 5 years have provided preliminary proofs that such nanosystem is possible to develop. However, as discussed earlier, there are many barriers to topical administration. Hence, mechanistic studies need to be performed to determine the biodistribution and elimination of nanomicelles following topical

administrations. Properties of nanomicelles such as size, shape, surface charge, rigidity of structure need to be studied in details to develop an efficient nanocarrier system that can safely deliver API at therapeutic concentration effectively.

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Figure 1.
The anatomy of the eye and various routes of administrations.

Figure 2.

Schematic illustration of formation of spherical micelle and drug encapsulation. Above critical micelle concentration the amphiphilic molecule (Surfactant or polymer) self-assemble to form core-shell structure depicted above. Hydrophobic drug (black dots) may be encapsulated during or after the micelle formation. The hydrophilic segment (Blue color) could be water soluble polymer like PEG or charged group of surfactant. Hydrophobic segment (Gray color) could be water insoluble lipid or polymer chain.

Figure 3. Structures of various micelle forming amphiphilic polymers and surfactants. Corona forming hydrophilic part of amphiphilic molecule is shown as blue color.

Figure 4. Release profile of DEX from the F127 and F127/chitosan micelle systems under sink conditions at 25°C (mean $\pm$ SD, $n = 3$). (Reproduced with permission reference#23).

Figure 5.

Cumulative amounts of DEX released from human sclera in the release studies performed after the passive (open diamonds) and cathodal iontophoretic (closed diamonds) transport experiments of UMM. The results of the control after passive delivery (open triangles) are presented again for comparison. Data represent the mean and standard deviation, $n = 3$. (Reproduced with permission reference#38).

Figure 6.

Dexamethasone concentrations in aqueous humour after administration to rabbits. Circles are PHEA-PEG-C16 micelles; squares are dexamethasone suspension. Each data point corresponds to the mean dexamethasone concentration in ng/ml $\pm$ SEM determined in the aqueous humor at each sample time. *Student's t-test, $P < 0.05$. (Reproduced with permission reference#39).

Figure 7.
Schematic illustration of formation of PIC micelles.

Table 1

Summary of nanomicelle/Micelle systems investigated thus far for ocular drug delivery.

Polymer/Surfactant	Therapeutic agent	Size	PDI	Surface charge	Remarks	Reference
Pluronic F127	Pilocarpine	23.3 ± 0.5 nm (in DDI water), 30.3 ± 0.3 nm (in buffer, pH 7.4)	NR	NR	64% increase in AUC along with significantly prolonged miotic activity with nanomicelles.	53
Poloxamine (Tetronic® 1107)	α-Tocopherol	30–40 nm	0.475	NR	Micelles were stable for months at 4°C	54
Poloxamines (Tetronic® T908, T1107 and T1307)	Ethoxzolamide	Multimodal size distribution	NR	NR	Tunable drug release profile was achieved with mixed micelle system.	22
MPEG-hexPLA	Cyclosporin A	54 ± 1 nm	0.229 ± 0.008	NR	The polymer well tolerated in rabbits. In vivo transcorneal permeability was improved and nanomicelle formulation was significantly efficacious in preventing corneal graft rejection.	28, 55-57
mPEG-PDLLA	Prenzepine hydrochloride	PEG/PLA wt ratio	NR	NR	In vivo biocompatibility study in rabbits exhibited on significant toxicity for 9 months. Intraocular levels of Prenzepine hydrochloride following topical instillation were enhanced with nanomicelles.	58
		80/20				
		50/50				
		40/60				
Pluronic F127 with Chitosan	Dexamethasone	25.4 - 28.9 nm	0.39-0.54	+9.3 to +17.6 mV	In vitro permeability increased with increase in chitosan concentration. Improved in vivo bioavailability.	23
Pluronic F127 with Chitosan (0.3-0.8%)	metipranolol	123–232 nm	0.117-0.157	+6.1 to +9.2 mV	Pharmacological response significantly improved upon incorporation of chitosan.	59
Crosslinked micelles made of NIPAAm, VP, and MAA with MBA and TEGDMA as cross linking agents	Dexamethasone	300–450 nm	NR	NR	Micelles exhibited very high entrapment efficiency and bioadhesive properties. It also resulted in higher anti-inflammatory activity for an extended duration.	60
mPEG-PCL	Dexamethasone	28 nm	NR	0.135	Aqueous solubility of dexamethasone was increased up to 1.36mg/ml. The nanomicelle enhanced dexamethasone permeability across the excised rabbit sclera by 2.5-fold.	13
Crosslinked micelles made of NIPAAm, VP and AA having cross-linked with MBA	Ketorolac (free acid)	35 nm	NR	NR	A 2-fold increase in permeability was observed across excised rabbit cornea. Nanomicelles significantly improved In vivo ocular anti-inflammatory activity.	61

Polymer/Surfactant	Therapeutic agent	Size	PDI	Surface charge	Remarks	Reference
Quaternary ammonium palmitoyl glycol chitosan	Prednisolone	10-100 nm	NR	NR	A significantly higher aqueous humor levels were achieved with formulation following single topical instillation.	62
Flt1 peptide-hyaluronate (HA) conjugates	Genistein	172.0 ± 18.7 nm	0.25 ± 0.11	-23.4 ± 5.1 mV	A significant suppression of corneal neovascularization was observed in silver nitrate cauterized corneas of rats. The retinal vascular hyper-permeability was reduced in diabetic retinopathy model rats.	63
PEG-P(Asp)	FTIC-P(Lys)	50.7 nm	0.046	NR	PIC micelles specifically accumulated in CNV lesions following tail Injection in rat CNV model.	
PEO-PPO-PEO	Mechanistic study with model plasmid DNA with <i>lacZ</i> gene	155 ± 44 nm	NR	-4.4 ± 2.0 mV	Micelle significantly enhanced In vivo gene transfer efficiency to ocular tissues in rabbit and nude mice models. Endocytosis was delineated as major transport mechanism for micelles.	64
Polyethylene glycol 40 stearate	Cyclosporine A	200 nm	NR	NR	A significantly higher Cyclosporine A level in the cornea, conjunctiva, and lacrimal gland were found with micelles.	35
Sympatens AS	Cyclosporine A	9.7 ± 0.05 nm	<0.1	-0.4 ± 0.1 mV	Nanomicelles enhanced corneal levels of cyclosporine A following topical dose compared to Restasis®.	33
PHEA-PEG ₅₀₀₀ -C16	Dexamethasone alcohol	10-30 nm	NR	NR	Nanomicelle formulation enhanced in vivo bioavailability of dexamethasone alcohol in rabbits.	39
Sympatens AS and Sympatens ACS	Cyclosporine A	9-12 nm	<0.16	neutral	In the porcine in situ model (ex vivo), remarkably high cyclosporine A levels in the cornea were observed for the nanomicellar solution	65

NR= Not reported

mPEG-PDLLA = Methoxy poly(ethylene glycol)-poly(D,L-lactide)

mPEG-hexPLA= Methoxy poly(ethylene glycol)-hexylsubstituted poly(lactide)

mPEG-PCL= Methoxy poly(ethylene glycol)-polycaprolactone

AUC = Area under curve

NIPAAm = N-isopropylacrylamide

VP = Vinyl pyrrolidone

MAA= Methacrylate

AA = Acrylic acid

MBA = N,N'-methylene bis-acrylamide
 TEGDMA = Triethyleneglycol dimethacrylate
 FIT1 peptide = Sequence GNQWFI
 PHEA = Polyhydroxyethyl aspartamide
 PEG = Poly(ethylene glycol)
 FITC-P(Lys) = Fluorescein isothiocyanate-labeled poly-L-lysine
 PEG-P(Asp) = poly(ethylene glycol)-block-poly-K,L-aspartic acid)

Table 2

Pharmacokinetic Parameters for IOP Increase after Ocular Administration of 0.1% DEX in Commercial Eye Drops or 0.025% DEX in Micelle Systems Based on F127 or F127 and chitosan (Reproduced with permission reference#23).

Ocular Formulations	$t_{\max}$ (h) (a)	$t_{\min}$ (h) (b)	AUC (% Increase in IOP _t × h)	kel, app(h ⁻¹) (c)	AUC _{rel} (d)
F127/DEX in isotonic acetate buffer, pH 4.5	1.37 ± 0.21 *	10.15 ± 0.94 *	115.9 ± 8.31 *	0.189 ± 0.05 *	1.72
F127/DEX/ 0.015 (w/v) % chitosan in isotonic acetate buffer, pH 4.5	1.25 ± 0.23 *	11.23 ± 0.56 *	162.8 ± 11.23 *	0.148 ± 0.05 *	2.41
Commercial DEX (0.1, w/v, %) eye drops	2.26 ± 0.14	8.39 ± 0.38	67.5 ± 9.42	0.319 ± 0.06	1

a Time needed to achieve peak IOP increase.

b Duration of IOP increase response (the time interval needed for the IOP to return to its normal pretreatment value).

c Approximate values calculated from the slope $\ln$ (% increase in IOP)/ t for the terminal points of % increase in IOP_t versus t curves.

d Ratio of AUC to the value for the commercial DEX (0.1, w/v, %) eye drops.

* Statistically significant difference as compared with commercial DEX (0.1, w/v, %) eye drops ($p < 0.05$).

Table 3

CyA Levels (ng-eq/g) in the Ocular Tissues after Single Instillation (50 μ L) of Three CyA Formulations in the Rabbits post 24 h.

Ocular tissue	Aqueous-CyA	Emulsion-CyA	Oil-CyA
Lacrimal gland	1.00 $\pm$ 0.42	0.51 $\pm$ 0.13 ^{**}	2.17 $\pm$ 0.99
Harder gland	0.42 $\pm$ 0.15 ^{**}	0.27 $\pm$ 0.07 ^{**}	1.64 $\pm$ 0.30
Bulbar conjunctiva	27.83 $\pm$ 7.06	19.70 $\pm$ 6.18 [*]	59.07 $\pm$ 33.31
Corneal epithelium	4932.76 $\pm$ 2143.96 ^{**}	2662.46 $\pm$ 653.42	643.94 $\pm$ 150.51
Corneal stroma–endothelial	491.12 $\pm$ 114.00 ^{**}	201.64 $\pm$ 69.33 [*]	31.33 $\pm$ 7.02
Aqueous humor	1.45 $\pm$ 0.31	0.52 $\pm$ 0.15	Not Detected
Choroid–retina	1.54 $\pm$ 0.95	0.36 $\pm$ 0.08	0.92 $\pm$ 0.57
Iris–ciliary body	23.95 $\pm$ 8.47 ^{**}	10.07 $\pm$ 2.74	1.16 $\pm$ 0.27

* P < 0.05

** P < 0.01 vs. castor oil (Dunnett multiple comparison test) (Reproduced from reference#35).

Pharmacokinetic Parameters of 14C-voclosporin-derived radioactivity following a single or repeat (QD for 7 days), bilateral ocular administration of 14C-voclosporin in a mixed micellar formulation to female NZW rabbits (US8435544 B2). (Reproduced with permission reference#36).

Table 4

Ocular Tissue(s)/Fluids & Blood	C _{max} (ng-cq/g)			AUC (h*ng-cq/g)			T _{max} (h)		t _{1/2} (h)	
	SD	RD	Ratio	SD	RD	Ratio	SD	RD	SD	RD
Aqueous Humor	6	13	2.3	45	96	2.1	0.5	0.5	-	14
Choroid/Retina	48	76	1.6	472	897	1.9	1.0	2	23	-
Cornea	1203	3382	2.8	23166	54624	2.4	8.0	0.5	-	-
Iris/Ciliary body	20	119	5.8	382	1952	5.1	24.0	1	-	-
Lacrimal Gland	31	120	3.9	416	1109	2.7	2.0	4	-	6
Lens	4	26	6.7	47	356	7.5	24.0	0.5	-	-
Lower Bulbar	1810	2929	1.6	12029	16585	1.4	0.5	0.5	10	7
Conjunctiva Lower Eyelid	20814	41635	2.0	207630	358791	1.7	1.0	0.5	-	-
Nictitating membrane	1716	2468	1.4	12135	15964	1.3	0.5	0.5	7	8
Optic Nerve	83	164	2.0	569	1805	3.2	0.5	0.5	-	16
Sclera	223	367	1.6	2646	3825	1.4	0.5	0.5	-	16
Submandibular	74	120	1.6	893	1190	1.3	2.0	2	-	-
Lymph Node Tear	20246	30904	1.5	168259	230878	1.4	0.5	0.5	-	7
Upper Bulbar	2235	3170	1.4	14782	19944	1.3	0.5	0.5	7	7
Conjunctiva Upper Eyelid	9896	17500	1.8	114651	98656	0.9	1.0	0.5	-	4
Vitreous Humor	2	2	1	27	23	0.9	8.0	4	-	-
Blood	BQL	BQL	NC	NC	NC	NC	NC	NC	NC	NC

SD = Single dose;

RD = Repeat dose;

Ratio = Repeat dose/Single Dose.;

- = Insufficient tissue concentration to determine t_{1/2};

BQL = Below Quantifiable Limit (<0.1 ng/mL);

NC = Not calculated.