OCULAR DELIVERY OF ANTIFUNGAL AGENTS

1. Introduction
Fungal infections of the eye are less common than infections with bacteria or viruses, but are usually severe and may lead to loss of vision (Whitcher et al., 2001). There has been an alarming increase in fungal infections in recent past years. The major contributing factors are increase in population of immune-compromised patient, patients undergoing transplant and cancer patients receiving chemotherapy. Diagnosis of fungal infections may be delayed due to gradual onset of the symptoms and empirical treatment with antibacterials.

Ocular fungal infections have become the foremost reason of blindness and can lead to life-threatening condition (Levin et al., 1996). Number of patients with complete recovery are usually low and those recovered may have compromised vision (Mithal et al., 2015; Thomas and Kaliarmurthy, 2013). Estimates give varying figures, but as high as 6 million people may suffer from fungal eye infections (fungal keratitis, endophthalmitis), every year (FIT, 2013). Infections due to Aspergillus and Candida species are the most common and Cladosporium, Sporothrix, Cylindrocarpon, Penicillium, Rhizopus, Mucorales, Pseudallescheria, Histoplasma, Blastomyces, Chrysonilia, Coccidoides, Cryptococcus species and Bipolar Hawaneseii are among other causative fungi (Al-Badriyeh et al., 2010).

Ocular infections may involve the cornea (keratitis), the interior of the eye (endophthalmitis), or the orbit. It may occur following trauma (such as surgery) or systemic disseminated infection (Whitcher et al., 2001). Such ocular infections are sight threatening and are challenging to the ophthalmologist because it has tendency to mimic stromal inflammation, and its treatment is limited by the nonavailability of effective antifungal agent with appreciable penetration in ocular tissues (Thomas et al., 1987).

Fungi has resistant cell walls which provides them protection from immune attack enabling them to thrive in the eye (Narang et al., 2001).

2. Ocular physiology and fungal infection treatment
Ocular disease may lead to discomfort for the patient because of the fear of blindness or facial disfigurement. After cancer, loss of vision is the most dreaded disease in general population (Kaur and Kakkar, 2014). Being an easily accessible organ topical delivery to the eye is highly patient compliant route of application and delivery. It is associated with several advantages. Firstly the drug effect is localized to the required
site with minimum amounts reaching the systemic circulation and eliciting associated side effects. Secondly, drug concentrations that are hard to achieve systemically can be achieved upon topical application in the ocular tissues. Thirdly, topically administration of drug avoid hepatic metabolism and last but not the least topical administration is convenient, simple, and painless.

Topically applied drugs however to some degree must be soluble in the aqueous tear fluid. They must also possess some lipid solubility in order to penetrate the lipophilic corneal epithelium and endothelium. However, hydrophilicity is equally essential for passage across the aqueous stroma and finally through single cell thick endothelium into the aqueous humour (Ahmed et al., 1987; Wang et al., 1991). In other words, a successful aqueous eye drop formulation of a drug must be both water-soluble (i.e. hydrophilic) and lipid-soluble (i.e. hydrophobic) (Loftsson and Stefansson, 2002). Usually, that water-soluble drugs are delivered through topical administration in an aqueous solution (Lang, 1995), and water-insoluble drugs are administered topically as an ointment or aqueous suspension. Contact time of the topically applied drugs with the eye surface is another limiting factor. A continuous tear secretion results in wash out of applied therapeutic agents resulting in low ocular bioavailability (Chrai et al., 1973). Pulse entry is a common, yet highly undesirable, pharmacokinetic characteristic, associated with eye drops (Shell, 1984). The initial high drug concentration found in tears, followed by a rapid decline, thus posing a potential risk of toxicity, and the subsequent drug loss, suggests repeat dosing at frequent intervals. A number of factors, namely rapid tear turnover and the resulting precorneal loss, induction of tear flow due to irritation caused by the drug preparation, as well as the relatively large volume of the administered eye drop (~50 µl versus 7 µl of corneal tear film) lead to a high rate of lacrimal drainage. In accordance with these anatomical and physiological constraints less than 5% of a topically applied drug is absorbed through the cornea into the eye (Loftsson and Jarvinen, 1999). Due to fast elimination rate the precorneal half life of drugs applied by the pharmaceutical formulations is considered to be about 1-3 min (Zimmer and Kreuter, 1995). Tear turnover and drug binding to tear fluid proteins are additional precorneal factors that contribute to the poor ocular bioavailability of many drugs when instilled in the eye in the solution dosage form. This indicates the need for an ocular drug delivery system which has the convenience of a drop (better compliance) but will serve as slow release depot (Nagarsenker et al., 1999).
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Nasolacrimal drainage is another factor contributing to precorneal drug loss and poor ocular bioavailability. It is also the major route of entry into the circulatory system, for drugs, that are applied through topical administration (Urtti and Salminen, 1993) resulting in unwanted side effects and toxicity. Bioavailability can be increased by reducing the effects of ocular drainage and increasing corneal residence time. To increase corneal residence time, various polymers, have been used to increase the viscosity of eye drops and the bioadhesion of solutions instilled into the eye (Kaur and Smitha, 2002). Colloidal dosage forms, such as liposomes, nanoparticles, nanocapsules, microspheres, microcapsules (Bourlais et al., 1998), and microemulsions (Vandamme, 2002) are thus advocated for use as drug reservoirs and can therefore prolong drug residence time on the cornea, consequently prolonging the drug’s pharmacological activity.

3. Ocular mycosis

The two types of ocular mycoses viz. keratitis and endophthalmitis, are usually reported. Same are discussed at length in the subsequent sections:

3.1 Keratitis

Fungal keratitis is one of the major causes of ocular fungal infections (See et al., 1998) accounting for >50% of proven cases (Hagan et al., 1995). Corneal fungal infection is represented by defect and in corneal epithelium and inflammation in the cornea. If not treated it may lead to scarring of cornea and ultimately to blindness. According to the World Health Organization, corneal diseases are major cause of blindness which in overall importance is second only to cataract (Whitcher et al., 2001). It is the second most common cause of vision loss in the developing countries (Chowdhary and Singh, 2005; Hoflin-Lima and Roizenblatt, 2002; Rosa et al., 1994; Williamson et al., 1968). It is a major blindness causing disease in Asia (Sharma et al., 1993). India has 2 million corneal blind people, accounting to a quarter of world’s total corneal blindness. However, according to the Andhra Pradesh Eye Disease Study, found that 0.12% of the population is corneal blind, which when extrapolated to the whole country gives a huge figure of 6.5 million (Dandona et al., 2001). Corneal infections due to trivial trauma is a major cause of blindness (Gonzales et al., 1996; Whitcher and Srinivasan, 1997).

Fungal keratitis often occurs in developing countries, accounting to >50% of keratitis (Galarreta et al., 2007). In South East Asia and India, fungal keratitis accounts for 40 to 50% of microbial keratitis, in contrast to the western countries (Dandona et al., 2001).
In different Indian studies, fungal keratitis represents 30-62% of culture positive corneal fungal infections (Bandyopadhyay et al., 2012). Sharma et al. (1993) reported that 44% of total corneal ulcers are because of fungal infections. In South India corneal fungal infection represented 30-40% of all cases of culture positive keratitis (Bharathi et al., 2003). Lalitha et al. (2015) reported increase in fungal keratitis cases as compared to bacterial keratitis from 2002 to 2012 in South India.

Similarly an incidence of: 32% is reported in East India (Dutta et al., 1981); 38.9% in West India (Verenkar et al., 1998); and 32-39.8% in South India (Gopinathan et al., 2002, Srinivasan et al., 1997) while lower in North India (7.3%) (Chander and Sharma, 1994). This regional difference could be because fungal infections are more common in the tropical and subtropical regions as compared to the temperate region. This may be due to hot and humid weather and large agriculture-based population (Gopinathan et al., 2002; Rosa et al., 1994) of tropical region (Bandyopadhyay et al., 2012; Bharathi et al., 2003). Similarly the prevalence of fungal keratitis is low in temperate zones in western world such as Northern United States and Britain (Asbell and Stenson, 1982; Lalitha et al., 2008; Thew and Todd, 2008). The incidence of corneal fungal infections is also high in countries such as Nepal (17%), Bangladesh (36%), Ghana (38%), and South Florida (35%).

Several reports indicate Aspergillus species to be a major cause of fungal keratitis from India (Chander and Sharma, 1994; Kotigadde et al., 1992; Mohanty et al., 1984) and Candida species in other parts of the world (Musch et al., 1983; Ormerod et al., 1987; Tanure et al., 2000). Candida albicans is the second most common cause in India (Saha et al., 2009). Candida infection is more common in temperate climate of the West compared to the tropical climate of East. Factors other than climatic conditions, such as altered local defense mechanisms and immune suppression may also be responsible for the higher incidence of Candida infection in some studies compared to others (Saha et al., 2009). Aspergillus species is the most common cause of corneal fungal infections, because it is resistant to hot and dry conditions (Leck et al., 2002).

Fungi are not able to penetrate healthy corneal epithelium, it needs an epithelial injury or defect to penetrate and establish a "foot hold" however, once within cornea, they are able to proliferate very fast. Candida is the most common fungi that invades
through a pre-existing defect/injury; while the filamentous fungi infections mostly occur post-trauma (Kaur et al., 2008b). Risk factors for keratitis are trauma especially with vegetable matter, contact lens, topical use of steroids, traditional eye remedies and ocular conditions such as dry eye, bullous keratopathy and lagophthalmos. The most common systemic predisposing causes are diabetes mellitus and immunocompromised status of the patient (Landers et al., 2010; Whitcher et al., 2001; Whitcher and Srinivasan, 1997). Further, fungi are also present in the microbial flora of the conjunctiva of eyes in approximately 3–28% (Arcieri et al., 2007; Sehgal et al., 1981) of the population which, in case of trauma, steroid administration, or diseased corneas can invade the cornea to cause keratitis. Available reports suggest that corneal fungal infections are common in older population (51-60 years) (Chander and Sharma, 1994; Rosa et al., 1994; Tanure et al., 2000). However, reports (Chowdhary and Singh, 2005; Kaur et al., 2008b) from Indian sub population indicates that the younger population who are often the breadwinners of the family in the age group of 31-40 years (~36%), followed by 21-30 years (~31%) were more common groups contacting the disease. Thus blindness of this age group can be of great economic set back to the family of the patient (Kaur et al., 2008b). Furthermore, Bharathi et al. (2003) reported that younger population (21-50 years) than to those >50 years of age, are more commonly afflicted with bacterial corneal infection. The ocular fungal infections may not follow the same therapeutic principles as the ones for systemic infections (O'Day, 1987), thus indicating need for antifungal eye formulations.

### 3.2 Endophthalmitis

Endophthalmitis is sight-threatening intraocular inflammation of the interior eye that may be due to an infectious organism (which may be bacterial, fungal or viral). This inflammation of internal ocular tissues (vitreous, retina, choroid), is often observed following trauma (post-traumatic), surgery (postoperative), or an infection in the body (endogenous). It is characterized by a decrease in visual acuity, severe pain and, if not treated, may lead to loss of vision. Cataract surgery may lead to more postoperative endophthalmitis cases than any other ocular surgery (West et al., 2005). The coagulase-negative *Staphylococcii* is the most common pathogen for postoperative endophthalmitis cases (48-70%). In comparison to post-traumatic or post-operative endophthalmitis, endogenous endophthalmitis is rare, 2-8% of all the reported cases (Jackson et al., 2003; Romero et al., 1999). Fungal pathogens are reported to be the
cause of nearly 50% of all the endogenous cases, and *C. albicans* (75-80% of the fungal cases) being the most common among them (Okada et al., 1994, Romero et al., 1999) followed by *Aspergillus* species (Essman et al., 1997).

Narendran et al. (2008) reported *Candida* to be the leading causative agent for fungal endophthalmitis. However, Chakrabarti et al. (2008) reported *Aspergillus* to be the main causative agent of fungal endophthalmitis in a study conducted at Postgraduate Institute of Medical Education and Research, Chandigarh, India.

The risks associated with ocular tissue damage present a challenge in selecting a suitable drug delivery vehicle that can deliver the optimal therapeutic dose while minimizing further loss of tissue-function efficiency.

Current approaches to control endophthalmitis include intravitreal amphotericin B (AMB; 5–10mg/0.1ml), voriconazole (VCZ), fluconazole (FCZ) (Chakrabarti et al., 2008), and systemic antifungal therapy and pars plana vitrectomy (Riddell et al., 2011). AMB achieves poor concentrations in the posterior eye segment but concentrations achieved with FCZ and VCZ are high (Riddell et al., 2011). FCZ is considered to be the most probable choice for treatment of endophthalmitis (Riddell et al., 2011). AMB, which is often associated with organ toxicity at therapeutic levels e.g. irreversible kidney toxicity, increased liver enzymes, hepatotoxicity, cardiac arrest, skin reaction and electrolyte imbalance is not generally preferred. VCZ, at the doses used in the current modes of treatment, is associated with several side effects including transient visual disturbances, sepsis, diarrhea, peripheral edema, respiratory disorder, and elevated hepatic enzyme levels. However, by reducing the dose administered, the risks and side effects associated with VCZ can be reduced (Leroy, 2010).

### 4. Antifungal agents and need for drug delivery

Antifungal drugs are broadly classified into four main categories which are- polyenes, azoles, and echinocandins (Al-Badriyeh et al., 2010). The antifungal agents like clotrimazole, econazole (ECZ) and flucytosine which were commonly used in 1970s and early 1980s for management of ocular fungal infections are not recommended now due to their poor efficacy and bioavailability in ocular tissues (Thomas, 2003).

The efficacy of antifungal drugs dependent on concentration being achieved in the ocular tissues which further depends on various other factors including molecular weight, dose and penetrability of the drug, its period of contact with the target site and route of administration of the drug (Nagarsenker et al., 1999). In order to achieve
therapeutic levels in the target ocular tissues, frequent application of eyedrops is a usually recommended, however this is laborious, may cause poor patient compliance and irritation. The duration of contact between the drug and the ocular tissues (cornea and conjunctiva) can be increased by the use of ointments and subconjunctival injection. However, miconazole (MCZ) is only available as ophthalmic ointment(Kaur et al., 2008b).

The prime objective of the management of ocular fungal infections is to maintain vision of the patient which depends on early identification and appropriate therapy with efficient administration(Manzouri et al., 2001). Most of the topical medications used for treatment of mycotic keratitis are custom-formulated from parenteral antifungal medication eg VCZ and AMB.

Of the aforementioned antifungal agents, AMB, natamycin (NTM), FCZ, and MCZ have been routinely used to manage ophthalmic mycosis for considerable span of time. The available antifungal therapy for ocular infections is inadequate due to poor ocular tissue penetration after topical and systemic administration, narrow antifungal spectra and limited clinical/therapeutic success achieved with the drugs. Compared to antibacterials, antifungals have a lower efficacy due to their mechanism of action (usually fungistatic, with fungicidal action being dose dependent), lower tissue penetration, and the indolent nature of the infection (Müller et al., 2013).

In order to overcome the therapeutic failure due to topical administration of the antifungal agent, additional systemic antifungal agent is usually recommended by the physician(Thomas, 2003), which enhances the cost of treatment and risk of adverse events. This has led to the use of newer antifungal agents like VCZ, posaconazole (PCZ), and caspofungin, or fabrication of the currently used drugs, for which the safety profile has been established, in a suitable delivery system (Al-Badriyeh et al., 2010) (Fig. 1).

The retina and choroid are more vascular than the vitreous cavity and vascular compartments are separated from the other intraocular structures by the blood-ocular barrier making systemic route of administration inefficient. But infections confined to the choroid can be efficiently treated with systemic antifungal drugs as it is not protected by these barriers(Riddell et al., 2011). Hence, for retinal and vitreal infections, local intraocular injection along with vitrectomy may be required (Brod et al., 1990).
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Most regions of the eye are relatively inaccessible to systemically administered drugs hence the topical drug delivery remains the preferred route in these situations. The antifungals being highly toxic, their systemic administration is a concern for organs toxicity. Furthermore, several antifungals are associated with drug-drug interactions which is again a concern for systemic administration if the patient is on other interfering drugs too. Topical route thus seems to be convenient alternative. This is well accepted route of drug administration which can also address issues related to poor penetration, limited antimicrobial spectrum and high toxicity of the currently available antifungal agents (Habib et al., 2010).

As discussed before antifungal efficacy depends on molecular weight and and penetrability of the drug in the ocular tissues (Manzouri et al., 2001). Antifungal drugs with molecular weight>500 Da e.g. AMB (924.10 Da), NTM (665.75 Da), and ketoconazole (KTZ; 531.44 Da) can scarcely penetrate an intact corneal and conjunctival epithelium because the generated large force of friction reduces diffusion (Manzouri et al., 2001). The diffusion is also dependent on solubility of the drug in lipid-rich ocular tissue. The penetration of the antifungal drugs with intermediate molecular weight like MCZ (416.12 Da) or FCZ (306.30 Da) is probably limited by the second factor. The lipid soluble drugs (lipophilic) e.g. itraconazole (ICZ) can efficiently traverse the lipidic epithelial and endothelial layers and blood-aqueous barrier; water soluble drugs (hydrophilic) are capable to traverse corneal stroma; and the biphasic drugs (having lipid and water solubility) can penetrate all the corneal layers (Friedberg et al., 1991). Furthermore, the penetrability of the drug also depends on the integrity of the corneal epithelium as it may be compromised due to the fungal infection (Thomas, 2003).

There may be significant differences within a class of antifungal drugs. For example, the lipophilicity (log D vary from 0.5 to 5.0), and plasma protein binding (ranges from 12% to 99%) for antifungal drugs. These physicochemical properties determine the rate and extent of tissue penetration and bioavailability within a tissue, organ, or fluid (Andes, 2003; Muller et al., 2004).

A compound with an “intermediate” lipophilicity, volume of distribution, and plasma protein binding (e.g., VCZ and FCZ) is predicted to distribute into aqueous and vitreous humor (Hariprasad et al., 2004; Spriet et al., 2009; Tod et al., 1997) of animal and/or human eyes, with and without endophthalmitis, at concentrations approximately 40-100% of those observed in serum and plasma. In contrast, more
lipophilic compounds (such as ICZ and PCZ) with much larger volumes of distribution, tend to penetrate preferentially into tissues with high lipid content, and often exhibit tissue/plasma concentration ratios that exceed 1. Despite this, they may not necessarily penetrate well into organs such as brain, prostate, and the eye (Felton et al., 2014).

Though new antifungal drugs seem to be promising yet loopholes still persist for efficient delivery of the agent. This has paved the way for development of suitable antifungal preparation with the use of novel drug delivery systems.

Fig. 1 Need for novel drug delivery systems to effectively deliver antifungal agents to the eye.

In this context, different antifungal drugs and delivery systems promoted for their most effective use, as are discussed in the subsequent section.

4.1 Polyenes

Polyenes still form an important class of ophthalmic antifungal therapy. They cause disruption of the fungal cytoplasmic membrane by directly forming bonds to a sterol distinctive to fungal cytoplasmic membrane, ergosterol (O’Brien, 1999). Although the
efficacy is dose dependent; however, beyond a certain dose it may affect human cytoplasmic membrane leading to its toxicity. Nystatin has not been used to treat eye infections for several decades due to its low tissue penetration, toxicity, and reports of inducing resistance (Reddy et al., 1982). However, currently AMB and NTM remain as the primary drugs in this class for the treatment of fungal eye infections (Ganegoda and Rao, 2004).

4.1.1 Natamycin (NTM)
NTM is the only Food and Drug Administration (FDA) approved antifungal drug available as eye drops (5% suspension) (Hariprasad et al., 2008; O’Brien, 1999). It has broad spectrum of activity against Candida, Penicillium, Fusarium, Acremonium, Aspergillus and Lasiodiplodia (O’Brien, 1999). It is also used as a pesticide and as a preservative in the food industry.

NTM has low water solubility (Thomas, 2003) hence is available in suspension form. However, it is stable in this form and is able to adhere efficiently to the ocular surface (Shukla et al., 2008). Although the suspension is viscous but is safe for ocular use without causing any irritation or damage to cornea. NTM is required to be instilled in the conjunctival sac at hourly or two-hourly intervals for 4–6 weeks to cure mycoses (Shukla et al., 2008).

It poorly penetrates the cornea and conjunctiva after topical application due to its high molecular weight (635Da) (Kaur et al., 2008b). As a result ineffective drug levels are achieved in the cornea and aqueous humor, hence its usefulness is limited to the treatment of superficial infections. Due to a low corneal penetration, therapeutic success with NTM requires its chronic administration averaging 39 days (Jones et al., 1970). Epithelial debridement is recommended as an adjuvant therapy so that higher concentrations can be achieved in the corneal stroma. This provides a greater adherence of the drug to the de-epithelised surface (Dong et al., 2012). NTM is still the first line therapy for fungal keratitis in several countries (Rosa et al., 1994), particularly for keratitis due to Fusarium (Müller et al., 2013). However, certain authors have shown that about one third of Fusarium infections do not respond to NTM (Jones et al., 1970, Müller et al., 2013). In such cases, NTM should be replaced by or associated with another drug (Müller et al., 2013). The applicability of topical NTM is compromised by corneal settling of the drug after topical administration where degradation occurs (Hariprasad et al., 2008). Subconjunctival administration is discouraged due to serious complications, such as scleritis and conjunctival necrosis.
There are no reports of administration of NTM through other routes (intracameral, intravitreal, intrastromal, or systemic) (Müller et al., 2013).

Being very poorly soluble, it is tough to incorporate it into a delivery system and only a few reports exist to this effect. Rajasekaran et al. (2010) developed ocusert of NTM comprising of Eudragit and hydroxypropyl methul cellulose which provided drug release over a period of 23h. Phan et al. (2013) prepared silicone hydrogel contact lens showing slow and sustained release of NTM for extended period. Lecithin/chitosan nanoparticles were prepared for NTM with a particle size of 213 nm and entrapment efficiency of 73.57% for mucoadhesive effect and enhanced bioavailability. The developed system was confirmed to be non-irritant (Bhatta et al., 2012).

4.1.2 Amphotericin B (AMB)

The drug name is derived from its amphoteric properties (soluble in extreme pH, both acidic and basic). AMB has long molecules that, when infused, coalesce into a colloid. Its action is primarily fungistatic, with fungicidal action which depends on concentration achieved in the ocular tissue (Khoo et al., 1994). Maximum activity is seen at pH ranging between 6.0-7.5. It has an excellent spectrum of activity against a variety of fungi including Candida species, Aspergillus species, Penicillium marneffei, Criptococus species and the causative agents of mucormycosis. It is also effective though, to a lesser extent, against the Fusarium species however no antibacterial activity is associated with it (Müller et al., 2013).

Systemically administered AMB is unable to penetrate the ocular tissues to reach therapeutic levels in the cornea, aqueous or vitreous humor (Kaur et al., 2008b). Furthermore, severe side effects associated with its use viz., nausea, chills, headaches, moderate anaemia, gastrointestinal cramps, anorexia, diarrhea, fever, local thrombophlebitis at the infusion site and vomiting, (Thomas, 2003), often discourage systemic administration (Müller et al., 2013).

AMB may be instilled by subconjunctival, intrastromal, intravenous, intracameral, intravitreal as well as topical for management of ocular fungal infections (Kaur et al., 2008b).

For intravenous infusion of AMB, a 0.1 mg/ml of solution in 5% dextrose is used. AMB should not be diluted in saline solution, as it may aggregate into a colloid which can reduce its bioavailability (Müller et al., 2013). The daily recommended dosage of AMB is 1 mg/kg body weight and a smaller dose may be ineffective. Renal toxicity is
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estimated to occur in almost 80% of patients receiving intravenous AMB (Panda et al., 1998).

However, for invasive fungal infections of the orbit intravenous AMB is still the preferred treatment option available (Kaur et al., 2008b, Thomas, 2003). Therefore a large dose may be required in case of intravenous administration due to poor penetration of AMB however, it may lead to renal dysfunction (Thomas, 2003), as mentioned before. Therefore, topical formulation of AMB is extemporaneously formulated from parenteral preparation in concentration of 0.15% (Bhartiya et al., 2007, Thomas et al., 1987). Topical administration in concentrations of 1.5 to 5 mg/ml is the treatment of choice for ophthalmic mycoses. The product has to be prepared by dilution with distilled water from the intravenous formulation (Fungizone™ - Bristol-Meyers Squibb, New York) diluted in distilled water. It is used at hourly intervals at the beginning of treatment, and then repeated every 4 hours after the therapeutic response starts appearing. Periodic debridement of the corneal epithelium is recommended during treatment, because the molecule’s large size hinders its penetration into the cornea if the epithelium is intact. After topical administration of AMB in rabbits whose corneal epithelium had been removed, therapeutic levels were reached in the corneal stroma. However, in corneas with intact epithelium, concentrations were low or undetectable (O'Day et al., 1986, O'Day et al., 1984, Qu et al., 2010).

In literature AMB has been reported to be used topically in form of ointment in management of of ophthalmic mycoses especially by Candida species (Hirose et al., 1997; Rosa et al., 1994; Tanure et al., 2000). However, contrasting claims on ocular safety are available, with 0.5% ointment being reported to produce conjunctival irritation (Rosa et al., 1994) while 2% ointment was well tolerated (Hirose et al., 1997).

Subconjunctival administration can be used in patients with low adherence to treatment, but the former is limited due to reports of conjunctival necrosis, scleritis and scleral thinning (O'Day, 1987; O'Day et al., 1991). Intracorneal administration, on the other hand, provides better results. There are few reports of complications with this route of administration, however it does provide higher and more sustained corneal concentrations than topical or intracameral administration. Several cases of keratitis unresponsive to topical treatment are successfully resolved after intrastromal administration (Garcia-Valenzuela and Song, 2005; Qu et al., 2010).

The current formulation of AMB eye drops (Fungizone®) contains deoxycholate, necessary to solubilise the highly hydrophobic AMB (Morand et al., 2007). However, instillation of drops containing deoxycholate is painful and leads to poor compliance and may aggravate symptoms, especially when direct intravitreal injection of AMB-deoxycholate is used for treating fungal endophthalmitis. Lipid formulations of AMB have been developed to overcome the retinal and systemic toxicity of conventional AMB when doses of AMB are required as in the treatment of zygomycosis (Walker et al., 1998). These formulations include AMB-lipid complex, which comprises of drug complexed to two phospholipids, dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol (Chapman et al., 2003) and the other being AMB colloidal dispersion. Latter combines cholesteryl sulfate and AMB (molar ratio 1:1) in disk-like array and diameters ranging from 120-140 nm) and dispensed lyophilized form (Moses et al., 1998).

Retinal damage and vitreal opacity associated with AMB administration, is reduced when lipid formulations, either “in-house” or commercially available such as AmBisome®, are used instead of AMB-deoxycholate in rabbit models. Localization of the drug inside the phospholipid bilayer is proposed to limit contact with epithelial cells resulting in reduced toxicity (Barza et al., 1985, Cannon et al., 2003, Tremblay et al., 1985). Thus, a combination of reduced toxicity, a longer persistence at the site of action and a higher AMB concentration would considerably increase the therapeutic index for the antifungal in the treatment of fungal keratitis (Morand et al., 2007). Tremblay et al. (1985) and Liu et al. (1989) have demonstrated reduced toxicity of liposomal formulation of AMB upon intravitreal injection. AMB deoxycholate (1 mg/kg of body weight/day), AMB lipid complex (5 mg/kg/day), or liposomal AMB (5 mg/kg/day) was given intravenously to rabbits as a single dose or as repeated daily doses for 7 consecutive days after induction of unilateral uveitis by intravitreal injection of endotoxin. Higher drug concentrations were achieved with liposomal AMB both in the aqueous and vitreous humor of inflamed eyes, suggesting a potential usefulness of liposomal AMB formulation in the adjuvant treatment of fungal endophthalmitis (Goldblum et al., 2002).

Payne et al. (2010) have reported retinal toxicity of AMB deoxycholate in rabbits with >10μg of dose; an earlier report demonstrated retinal damage at even 1μg (Souri and Green, 1974). Further, on comparing retinal safety of AMB-lipid formulations to AMB deoxycholate (Cannon et al., 2003, Tremblay et al., 1985) in rabbits, a dose-
dependent toxicity to the retinal ganglion cells was established and liposome formulation being least toxic (Cannon et al., 2003).

Another study conducted in primates reported reduced toxicity of liposomal formulation of AMB on intravitreal injection however, minimum toxicity was with AMB deoxycholate at dose of <30μg (Barza et al., 1985).

With the success of liposomes other nanocarrier systems have also been tried and reported to reduce toxic effectors improve bioavailability of AMB. Das et al. (2010) prepared positively charged Eudragit containing, AMB loaded nanoparticles, in order to increase residence time on cornea with particle size ranging from 134-290nm. Ibrahim et al. (2012) prepared polymeric nanoparticles of AMB (108-290nm) providing entrapment ranging from 45-67% having capability of slow and prolonged release of drug. Self-aggregated nanoparticles using an amphiphilic poly(lactic acid)-grafted-chitosan (PLA-g-CS) copolymer loaded with AMB (200nm) were also prepared and reported for ocular delivery. The prepared formulation showed sustained drug release with no sign of irritation after instillation. A 1.95-fold prolonged residence time at the ocular surface was shown by this formulation in comparison to free AMB solution, confirming the mucoadhesiveness of the nanoparticles (Zhou et al., 2013).

Collagen shields have also been reported to increase bioavailability of drug in ocular tissues with minimum number of applications. It is looks like a contact lens and is in dehydrated form which requires rehydration before use. The limitation associated with collagen shields is that prolonged exposure time may lead to toxicity as compared to eye drops which wash off fast with the tears (Friedberg et al., 1991). Literature reports effectiveness of collagen shields soaked in AMB for treatment of *C. albicans* keratitis (O’Brien, 1999; Schwartz et al., 1990) Schwartz et al. (1990) reported collagen shields soaked in AMB were found to achieve corneal AMB levels comparable to those achieved by hourly topical administration of drops.

**4.2 Azoles**

Azoles act by binding to a cytochrome P-450 fungal enzyme which is required for synthesis of ergosterol resulting in enhanced permeability of fungal cell membrane leading to inhibition of growth and death of the fungi.

Azoles are divided into two major classes — imidazoles, first to be introduced, followed by triazoles. Both class of compounds have similar antifungal spectra, but
triazoles have the advantages of being metabolised more slowly and exerting less influence on the metabolism of steroids in humans (Martinez, 2006, Müller et al., 2013). Imidazoles used often used in ophthalmology include MCZ, econazole (ECZ) and ketoconazole (KTZ). Among the first-generation triazoles, the most used are ICZ and FCZ. Second-generation triazoles were introduced into clinical practice in the past decade and include VCZ and PCZ (Müller et al., 2013).

Azole drugs have recently found their new use as antituberculars (antiTB) also. They have the ability to coordinate tightly to the haem iron in H37Rv P450s encoded by genes Rv0764c (the sterol demethylase CYP51) and Rv2276 (CYP121). However, they have an even higher affinity for Mycobacterium tuberculosis CYP121. This suggests that azole drug therapy may provide a novel antibiotic strategy against strains of M. tuberculosis that are resistant to convention antitubercular therapy with drugs like isoniazid (INH) and other front-line drugs (McLean et al., 2002). A report from Sun and Zhang (1999) showed that members of the azole class of antifungal agents had significant anti-TB activity in vitro. In addition, azole drugs are active against growing bacilli and, to some extent, stationary-phase bacilli too. Latter can help shorten the duration of TB therapy by killing a population of bacilli not readily killed by current antiTB drugs (Byrne et al., 2007). Bryme et al. (2007) reported that replacement of INH in a three-drug regimen with KTZ led to a greater reduction in the number of viable bacilli in infected animals. The in vivo activity of KTZ was evaluated in established pulmonary TB in the murine model, compared alone and in combination with isoniazid (INH), pyrazinamide (PZA) and rifampicin (RIF). KTZ alone exhibited little effect after short-term treatment, with a borderline bacteriostatic effect on spleen colony counts but not on lung counts. However, when KTZ was added in combination with INH, PZA and RIF, it significantly improved the treatment outcome in the lungs (compared with treatment with INH, PZA and RIF). The lowest numbers of bacilli in lungs were found in mice treated with KTZ, PZA and RIF.

Use of varius azoles along with different delivery systems employed for improving their efficacy for control of fungal infections of the eye are discussed below:

4.2.1 Miconazole (MCZ)

Systemic administration of MCZ though highly effective is not used commonly due to its cardiovascular and hepatotoxic side effects (Foster and Stefanyszyn, 1979; Thomas, 2003). However, it does show significant penetration into the ocular tissues (Foster and Stefanyszyn, 1979). The solution available for intravenous administration can
also be used for topical (1% w/v)(Foster et al., 1981) or subconjunctival administration (5 to 10 mg) (Fitzsimons and Peters, 1986).

In an experimental rabbit model, higher corneal levels were achieved with topical and subconjunctival administration of MCZ as compared to intravenous administration without toxicity with debrided cornea (Foster and Stefanyszyn, 1979). These results suggest that MCZ administered topically was effective in management of ocular keratitis(Thomas, 2003).

4.2.2 Econazole (ECZ)

ECZ, an imidazole with a molecular structure similar to MCZ, is used primarily in the treatment of superficial mycoses (Heel et al., 1978). Though used rarely in the treatment of eye infections, reports on its topical administration to treat fungal keratitis are available. In a controlled clinical trial comparing eye drops of ECZ 2% with NTM (5%), no statistical difference between the rates of therapeutic success in the two groups was observed. Both groups showed good results with no incidence of any adverse reactions(Prajna et al., 2003). However, ECZ is reported to be associated with ocular irritation (Thomas, 2003).

ECZ has wide spectra of activity against filamentous fungi majorly against Fusarium species (Thomas, 2003).

El Kasabgy (2014) developed supersaturated self-nanoemulsifying drug delivery systems (S-SNEDDS), thermodynamically stable, uniform preconcentrate of oils, surfactants and co-surfactants used to enhance the solubility of poorly soluble drugs by formation of o/w nanoemulsion upon dilution. S-SNEDDS (<15 nm)of ECZ have been developed to improve its ocular bioavailability.

In order to extend the contact time of ECZ on the surface of the eye, an ophthalmic ointment of ECZ has also been developed and reported(Fatohy, 2009).

4.2.3 Ketoconazole (KTZ)

KTZ, discovered in 1978, was the first effective orally absorbable antifungal drug. In 1980’s, it was the only FDA approved drug available (http://www.accessdata.fda.gov/Scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Label_ApprovalHistory#apphist) for the treatment of systemic fungal infections (Maertens, 2004). Good activity of KTZ against superficial and systemic infections caused by dermatophytes, yeasts, molds, and dimorphic fungi (Dixon et al., 1978; Heel et al., 1982; Thienpont et al., 1979), helped it gain importance; so as to be included in the World Health Organization (WHO) Model List of Essential Medicines for a long time (Janseen
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Pharmaceutica, 2014). However, from 1983 onwards, concerns were raised over the hepatotoxicity associated with the KTZ therapy (Janssen and Symoens, 1983; Yan et al., 2013). KTZ was also found to inhibit testosterone synthesis and adrenal steroidogenesis because of its nonspecific activity on mammalian P450-dependent enzyme (Daneshmend and Warnock, 1988; Loose et al., 1983). It also increases the risk of drug interactions through inhibition of the CYP3A4 enzyme; hence many drugs are contraindicated with KTZ (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Label_ApprovalHistory). Until 2013, KTZ was used as a main drug in the treatment of various superficial and systemic infections. In 2011, the National Agency for the Safety of Medicine and Health Products (ANSM), France, recommended suspension of KTZ saying that its risks outweighed its therapeutic benefits. This was followed by a ban in Europe and Australia (European Medicines Agency, 2013). Strict restrictions and cautionary advisements have also been imposed on the use of oral KTZ in USA and Canada (Health Canada, 2013; FDA, 2013), with use recommended only in serious/ life-threatening systemic fungal infections when no other alternative is available (Gupta and Lyons, 2015). Under such circumstances, the treatment must be cautiously monitored and should be interrupted in case elevated liver parameters or symptoms of abnormal liver function are observed. Although, more safe triazoles like FCZ have replaced KTZ for the treatment of systemic mycoses, topical formulations of the later are still in use in dermatology as they do not impose any systemic toxicity (FDA, 2013). Hence, the restrictions do not apply to topical formulations of KTZ in creams, shampoos, foams, and gels. Topical KTZ is used for treating ringworm, jock itch, athlete's foot, dandruff, and tinea versicolor. There are no known drug interactions with topical KTZ (http://www.medicinenet.com/ketoconazole/article.htm).

Although its penetration into the cerebrospinal fluid and urine is low, its penetration into ocular tissues is significant when used systemically. There are numerous reports of therapeutic success with oral KTZ with or without topical NTM or AMB in the treatment of fungal keratitis. Some authors suggest its routine use in all cases of fungal keratitis (Ishibashi, 1983, 1986; Thomas et al., 1987), but this is not supported by controlled studies. There are reports of cases treated exclusively with topical KTZ (10 to 50 mg/ml) (Torres et al., 1985), but other drugs have been shown to be superior in comparative studies.
Several invitro studies reportsusceptibility of keratitis causing fungal pathogens and ocular fungal isolates including *Aspergillus* species, *Candida* species and some *Fusarium* species were sensitive to KTZ (Maragon et al., 2004, Oji, 1982a, Therese et al., 2006). However, conflicting reports on efficacy of KTZ in animal models of ocular mycosis are available. In one report the topical application of KTZ (1%) was ineffective against superficial and deep keratitis induced by *C. albicans* (O'Day et al., 1983). In another report topical (5%) and oral (60mg/kg) KTZ were no better than placebo in reducing colony counts of *A. fumigates* in corneas, despite a moderate in vitro sensitivity to the drug (Komadina et al., 1985). In contrast several reports have shown that the topical application of KTZ was effective in the treatment of keratitis by *C. albicans* (Ishibashi and Kaufman, 1986) and by *Aspergillus flavus* (Oji, 1982a, b) by totally eradicating them. Also oral KTZ showed efficacy in the treatment of *C. albicans* keratitis model (Ishibashi and Matsumoto, 1984). The concentration of 1391 µg/g was obtained in debrided cornea following a topical administration (Hemady et al., 1992). Nor is the topical application of KTZ related with any major corneal and conjunctival toxicity (Foster et al., 1981, Oji, 1982a, b). Such contrasting reports could be assigned to the different experimental models used by various investigators, wide variation in the susceptibility of test organisms to KTZ and the use of different drug preparations (arachis oil, Cremaphor and petroleum ointment used as drug vehicles) in different concentrations varying from 1-5% (Hemady et al., 1992, Zhang et al., 2008).

However, reports on efficacy of KTZ in humans have been encouraging (Fitzsimons and Peters, 1986, Ishibashi, 1986). A case with effective treatment of fungal keratitis due to *Fusarium solani* and another due to an unidentified fungus, by administration of oral KTZ, was reported by Ishibashi (1983). Long-term use of oral KTZ was also reported to be effective in treating endogenous uveitis (Ramadan et al., 1997). Torres et al. (1985) found topical application of KTZ to be effective, without any significant toxicity, for treatment of patients suffering from *Aspergillus* and *Fusarium* infections. Sharma et al. (1993) reported successful cure of *Fusarium solani* keratitis in patients treated with KTZ in comparison to those treated with AMB, NTM and MCZ. NTM (5%), the most effective drug against most filamentous keratitis, is used in conjunction with oral KTZ when there is a danger of fungal invasion into the anterior chamber (Gonzales et al., 1996; Sonego-Krone et al., 2006).
An intravitreal dose (≤540 µg) of KTZ in dimethyl sulfoxide (DMSO) was reported to be safe for treatment of endophthalmitis (Yoshizumi and Banihashemi, 1988). However, papilledema was observed in a patient being administered 800 mg/day of drug for >4 months (Or et al., 1993). Further to this, KTZ showed good penetration in aqueous humor upon oral administration (Malecaze et al., 1987). Savani et al. (1987) detected KTZ in the cornea, aqueous humor and vitreous humor of rabbit after oral administration of 80 mg/kg and concentrations increased significantly after induction of ocular inflammation. Also KTZ was efficient to completely eradicate *C. albicans* endophthalmitis in rabbits even when treatment was postponed for 7 days. In an eye of patient undergoing vitrectomy for fungal endophthalmitis 6h after receiving oral KTZ, levels of the drug were determined to be 0.71 µg/ml in aqueous humor and 0.35 µg/ml in the vitreous humor (O'Day et al., 1985). In another study by Jones et al. (1981) the number of organisms were significantly reduced as compared to untreated group in Candida endophthalmitis rabbit model. Apart from being antifungal agent, oral KTZ also has a role in treatment of Acanthamoeba keratitis (Auran et al., 1987).

Inspite of its proven efficacy as a broad spectrum antifungal drug and suitability for topical use in treatment of keratitis, no ocular formulation of KTZ is marketed. This is due to its limited solubility and thus difficulty to formulate as eye drops. The antimycotic results are mainly dependent on drug concentrations achieved in the infectious site and antimycotic spectrum. To achieve high level of KTZ in the cornea or aqueous humor, it is desired to make it remain in the precorneal area for a longer time to penetrate into the cornea. A possible solution could be to encapsulate KTZ in a suitable delivery system for prolonged and effective results. Kakkar and Kaur (2011) prepared a nanosystem of KTZ for ocular delivery and confirmed its cytotoxicity potential and ability to target posterior eye.

Zhang et al. (2008) proposed hydroxypropyl-β-methylcellulose cyclodextrin (HP-β-CD) to complex with KTZ and administer the resulting solution as eyedrops for the treatment of keratitis. KTZ-HP-β-CD was found superior to free drug suspension as indicated by higher ocular bioavailability of the former.

### 4.2.4 Itraconazole (ICZ)

ICZ is a triazole which is highly lipophilic and found to be present in significant concentration in the lipidic tissues when administered orally (Savani et al., 1987). ICZ has activity against *Candida* and *Aspergillus* species; however, it is used rearely for
the treatment of fungal keratitis (Hariprasad et al., 2008) and is inactive against *Fusarium* species (Bhartiya et al., 2007; Hariprasad et al., 2008). A 1% suspension of ICZ, prepared in a commercial isotonic eye drop formulation containing methylcellulose, borax, boric acid, sodium chloride, and potassium chloride, though well tolerated, was not very effective in treating severe mycotic keratitis, perhaps due to insufficient corneal penetration (Thomas, 2003).

Although ICZ is generally considered to be safe however gastrointestinal upset is often reported (Rajasekaran et al., 1987) with its use. When administered orally it exhibits lower bioavailability, solubility and penetration into ocular tissues than other azoles (Kaur et al., 2008b; Rajasekaran et al., 1987). Topical use at a concentration of 10 mg/ml was not as effective as 5% NTM (Kalavathy et al., 2005). In vitro studies found that ICZ had a higher minimum inhibitory concentration (MIC) than AMB and NTM (Li et al., 2008; Rajasekaran et al., 1987), and associated drug resistance was detected among all analysed strains (Lalitha et al., 2007). ICZ was less effective than KTZ against *Aspergillus* species (Marangon et al., 2004). It is recommended to limit it to adjuvant therapy for the treatment of eye infections by yeasts (Müller et al., 2013).

### 4.2.5 Fluconazole (FCZ)

Formulated in 1981, FCZ is a stable, water-soluble, bis-triazole antifungal with low molecular weight (306 Da), low protein binding (10-20%) and low toxicity (Felton et al., 2014; Richardson, 1990; Savani et al., 1987). It is available as eye drops at a concentration of 0.3% w/v for the treatment of ocular mycoses. However it is reported to efficiently penetrate ocular tissues and fluids upon oral administration to rabbits (O'Day et al., 1990) with the concentrations in the cornea being comparable to that in the serum. Howsoever, oral administration is reported to induce thrombocytopenia, hypokalaemia and suppression of neutrophil function (Singh et al., 1993).

It has a short ocular half-life, of almost 15 min in the debrided eye and 30 min in the nondebrided eye (Yee et al., 1997) and exhibits low partition coefficient (log P 0.25) (Robert and Kalia, 2006; Kaur et al., 2012). The latter indicates poor permeability across the ocular epithelial membrane, ultimately leading to poor bioavailability and effectiveness.

The drug is well tolerated when injected intravitreally (Urbak and Degn, 1994). FCZ has become a drug of choice for treatment for ophthalmic mycoses due to its appreciable intraocular levels and ocular safety associated with it. It is generally recommended alone for treatment of chorioretinitis and in combination withintravitreal
therapy and/or vitrectomy (Riddell et al., 2011). Mochizuki et al. (1992) reported that systemic administration of 25 mg/kg of FCZ to the rabbit resulted in the vitreous levels of 20.63 μg/ml and did not evoke any significant changes in electroretinogram (ERG) up to the studied period of eight days. Schulman et al. (1987) conducted a toxicity study eight days after the intravitreal injection of FCZ at the dose of 100 μg/0.1 ml using biomicroscopy, ophthalmoscopy and electroretinography revealing no evidence of ocular toxicity.

A number of reports claim successful treatment of endogenous endophthalmitis induced by Candida species, with FCZ (Christmas and Smiddy, 1996). FCZ eye drops achieved good intracorneal therapeutic levels against strains of A. fumigatus in rabbits. When used at a concentration of 2 mg/ml, its penetration was better after epithelial scraping (Avunduk et al., 2003, Yee et al., 1997).

The penetration of 0.2% FCZ into corneas (with or without epithelial debridement) and the aqueous humors of New Zealand White rabbits (Yee et al., 1997) were assayed. The peak levels of 8.2±1.2 μg/g (debrided corneas) and 1.6±0.6 μg/g (non-debrided corneas) in corneas were noted after 5 min, and levels of 9.4±2.3 and 1.6±0.6 μg/ml, respectively, in aqueous humor were found after 15 min. A loading dose of a 20 μl drop/min for 5 min resulted in levels of 59.9±11.3 μg/g in debrided corneas and 32.4±1.9 μg/ml in the corresponding aqueous; this loading dose, followed by 1 drop (20 μl) every 1 or 6 h, resulted in lower levels (Yee et al., 1997). Therefore, Yee et al. (1997) recommended fabrication of FCZ in a sustained release ophthalmic formulation to avoid repetitive instillations.

Many experimental and clinical studies used FCZ in solution as eyedrops to treat deep keratitis. The eyedrops were administered every 30 min or 1 hr for 7–21 days depending on the severity (Behrens-Baumann et al., 1990; Goldblum et al., 2002; Schreiber et al., 2003). However patient compliance is an issue under such rigorous regimens. To address the requirement of high frequency of administration, FCZ-loaded liposomes are reported in as sustained release alternative. A prolonged corneal contact time and extended release of FCZ from liposomes can decrease the instillation frequency, onset and duration of recovery and healing from candidiasis. As liposomal preparations are known to provide a potentially longer contact time this leads to greater penetration of the FCZ (Behrens-Baumann et al., 1990; Yee et al., 1997; Yilmaz and Maden, 2005). FCZ loaded liposomal formulations were observed to be better than FCZ solution in Candida keratitis model which was attributed by the
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authors to the viscosity of liposomal formulation which is higher than that of the solution (Habib et al., 2010). The higher viscosity leads to an increase in residence time compared with the solution form (Bochot et al., 1998). FCZ-loaded liposomes were instilled four times daily in the first 3 days and then three times daily for the next period of treatment as in vitro results showed that liposomes had a sustained release action for 12 hr. In the rabbits treated with FCZ solution, 50% healing was observed in 3 weeks, whereas 86.4% healing was observed for animals treated with FCZ encapsulated liposomes (Habib et al., 2010).

Velpadian et al. (2006) compared the retinal toxicity of FCZ solution at the concentrations of 100, 200, 400 and 800 μg/0.1 ml as compared to liposome formulation of FCZ 100 and 200 μg/0.1 ml in rabbit eyes, upon intravitreal injection (Velpandian et al., 2006). The authors concluded that administration of FCZ solution caused photoreceptor disorientation and ultrastructural changes of the retina at the concentration of 100 μg/0.1ml or above. In contrast, liposomal formulation of FCZ did not show any retinal alteration up to concentration of 200 μg/0.1ml.

Gupta et al. (2000) found that the entrapment of FCZ into liposomes significantly slowed its clearance after intravitreal injection resulting in a higher FCZ concentration in the vitreous. The liposomes also showed a longer half-life (23.4 h) in comparison to free FCZ (3.08 h) (Gupta et al., 2000). Multilamellar vesicles of FCZ were one-fold more active against two strains of C. albicans than the corresponding free solution (Singh et al., 1993).

A biodegradable polymeric scleral implant containing FCZ was reported to be a promising intravitreal drug delivery system to treat fungal endophthalmitis (Miyamoto et al., 1997).

The thermoresponsive ophthalmic FCZ in situ gel comprising of poloxamer/chitosan (Lihong et al., 2014) was prepared to improve its residence time on the eye surface. Successful results have also been obtained with inserts and collagen shields. FCZ in polyvinyl alcohol: polyvinylpyrrolidone (K-30) 4:2.5 ratio was also evaluated (Rao et al., 2010). Complexation of FCZ with β-cyclodextrin suggested enhancing the solubility profile of FCZ and also permeability of the drug through cornea. This complex was then formed into an ocular insert prepared using hydroxypropyl methyl cellulose, a good film forming hydrophilic polymer. In vivorelease profile indicated that drug release was less compared to in vitreorelease, and no irritation or redness was
observed in the rabbit eye. FCZ ocuserts maintained the stability of the drug on storage with shelf life of 1.499 years (Ahad et al., 2011).

A biodegradable polymeric scleral implant containing FCZ was reported to be a promising intravitreal drug delivery system to treat fungal endophthalmitis (Miyamoto et al., 1997). Scleral implants loaded with 10, 20, and 30% doses gradually released FCZ over 4 weeks in vitro, while those with 50% doses released most of the drug in 1 week; implants with 30% FCZ that were studied in pigmented rabbits resulted in vitreous concentrations of FCZ (sustained for 3 weeks) sufficient to inhibit *C. albicans*.

Mohammed et al. (2013) reported FCZ loaded chitin nanogels which can be used for the treatment of corneal fungal infections. These nanogels were found to have controlled release pattern which is ideal for the continuous availability of FCZ over a longer period of time for an effective fungal treatment. Haemocompatible, cytocompatible FCZ loaded chitin nanogels showed very good cell uptake in human dermal fibroblast cells and penetration to the deeper sections of the porcine cornea with no signs of destruction or inflammation to corneal cells.

### 4.2.6 Voriconazole (VCZ)

VCZ acts by similar mechanism of action as that reported for first-generation triazoles. It is commercially available for oral and parenteral administration (Vfend® - Pfizer, New York). It is metabolised rapidly by the liver, hence liver enzymes need to be controlled during therapy. VCZ is a triazole antifungal agent with good penetrability into ocular tissues resulting in high bioavailability (96%) on oral delivery (Hariprasad et al., 2004). Similar to FCZ, it presents good gastric absorption and bioavailability (Hariprasad et al., 2008). When administered orally at a dose of 200 mg every 12 hours, VCZ reaches peak plasma concentrations after 2-3 hours. The drug has been extensively studied in the treatment of keratitis and endophthalmitis due to its good concentrations in several ocular tissues (cornea, vitreous and aqueous) (Al-Badriyeh et al., 2010; Müller et al., 2013). Hariprasad et al. (2004) found concentrations of VCZ in the vitreous and aqueous humors corresponding to 38% and 51% of plasma levels, respectively, after oral administration. Although the concentrations achieved in the vitreous were insufficient to treat infections by *Fusarium* species, the authors argue that the study was conducted in non-inflamed eyes, and that in the presence of inflammation a more permeable blood-ocular barrier would help increase the local concentrations of the drug.
VCZ treatment apart from being expensive therapy, may also lead to side effects and drug interactions (Hariprasad et al., 2004; Denning and Bromley, 2015). Among its side effects are visual disorders (blurred vision, change in colour perception and photophobia), which are present in about 30% of patients using the drug. Gao et al. (2003) reported its safety in an experimental model with rats, with no changes in electroretinography in doses up to 25mg/ml on intravitreal administration. Administered at a concentration of 1 mg/ml, VCZ was found effective in the treatment of keratitis caused by *Candida, Aspergillus, Fusarium, Scedosporium,* and *Paecilomyces* (Bunya et al., 2007; Lee et al., 2009).

Few case reports describe success to control keratitis unresponsive to topical NTM, using VCZ 50µg/0.1 ml (Prakash et al., 2008; Sharma et al., 2013). Three cases of unresponsive *Fusarium* keratitis were resolved after intracorneal VCZ. The authors suggested that direct injection of VCZ in the cornea increases its concentration above its MIC for *Fusarium* species. Sharma et al. (2011b) in a series with 13 patients, also suggested the use of intrastromal VCZ in refractory keratitis.

However, there are few studies which reflect failure of VCZ for treatment of fungal infections. In a multicenter randomized study VCZ was not found to be superior to NTM, with both groups having similar healing times and final visual acuity (Prajna et al., 2010). There are even reports of treatment failure with VCZ. Giaconi et al. (2006) reported two cases, a keratitis by *F. oxysporum* and another by *Colletotrichum dematium*, which were unresponsive to topical therapy with VCZ. Further, intrastromal VCZ injection was also found to be ineffective against filamentous fungi keratitis.

VCZ is a hydrophobic molecule which is not stable in aqueous medium (Davies, 2000; Hariprasad et al., 2004). Currently, VCZ is not available as topical formulation for treatment of ocular mycoses but is prepared from parenteral VCZ formulation (Lau et al., 2008). Parenteral preparation (*Vfend*, Pfizer) comprises of VCZ complex with sulfobutyl ether β-cyclodextrin sodium that enhances the solubility of VCZ and improves its corneal permeability (Davies, 2000; Sigurdsson et al., 2007). Cyclodextrin facilitates the formulation of the eye drop solution and improves its clinical properties. SBEβCD has already been used for ocular drug delivery, with success, at final concentrations ranging from 5 to 15% (Dupuis et al., 2009). Topical VCZ exhibits excellent penetration into the cornea, and high VCZ concentrations are reached in the anterior chamber after single dose application. It can be a promising...
agent to manage corneal fungal infections in future. However, Dupuis et al. (2009) found that the SBEβCD concentration in eye drop solution (1%w/v) prepared from marketed IV formulation of VCZ (Vfend; Pfizer) was equal to 160 mg/ml, leading to a hyperosmolar ophthalmic solution compared to the physiological osmolarity of tears. Clode et al. (2006) demonstrated effective penetration of VCZ eye drops in doses of 0.5%, 1.0%, and 3% solutions in horses, achieving a drug concentration of 1.43, 2.35, and 2.4 µg/ml, respectively in aqueous humor. Klont et al. (2005) reported a drug concentration in aqueous humor (3.2 µg/ml) to be 160% of that achieved in plasma (2.0 µg/ml) after 13 days of administration of VCZ eye drops (1%) along with oral VCZ therapy for Fusarium keratitis. The in situ gelling formulations of VCZ were prepared by using pluronic F-127 (PF-127) or with combination of pluronic F-68 (PF-68) and sodium alginate by cold method technique (Pawar et al., 2013). The antifungal efficiency against C. albicans and A. fumigatus, prolonged effect, and shelf life of 2 years was established for the developed system.

4.2.7 Posaconazole (PCZ)
PCZ is a second-generation triazole introduced into medical practice. It results from an improvement in the molecule of ICZ and is primarily indicated for the treatment of invasive fungal infections in onco-hematological patients. It is available only as an oral suspension (Noxafil™ - Schering-Plough, Kenilworth, New Jersey). Gastrointestinal complaints are the only adverse effects reported to date (Ullmann et al., 2006). In vitro and in vivo studies show that PCZ has a broad spectrum activity against Candida species, C. neoformans, Aspergillus species, and Fusarium species and it is was effective against most agents resistant to ICZ (Cuenca-Estrella et al., 2006; Torres et al., 2005). Together with VCZ it had the lowest MIC against multiple agents (Lalitha et al., 2007). Few reports are available concerning the efficacy of PCZ for treatment of ophthalmic mycoses. In a series of three cases of Fusarium keratitis progressing to endophthalmitis unresponsive to treatment with oral and topical VCZ, a rapid therapeutic response to PCZ was observed (Tu et al., 2007). Sponsel et al. (2002) described a case of keratitis by Fusarium solani resistant to AMB and NTM but successfully cured with oral PCZ and/or topical use (100 mg/ml prepared from an oral solution).

4.3 Echinocandins
Echinocandins are semisynthetic lipopeptides that inhibit the synthesis of glucan in the fungal cell wall through non-competitive inhibition of the enzyme D-glucan synthase, an enzyme involved in fungal cell wall synthesis, causing osmotic imbalance and cell lysis (Beauvais and Latge, 2001). Because mammalian cells lack a cell wall, it also represents an ideal and specific target for antifungal therapy. Echinocandins exert antifungal activity against Candida and Aspergillus species (Petraitis et al., 2002).

This class of drugs includes caspofungin, micafungin and anidulafungin. Used in yeast infections, echinocandins have rapid fungicidal action against most Candida species, but not against Cryptococcus, Rhodotorula and Trichosporon. Echinocandins have fungistatic action against some filamentous fungi such as Aspergillus, but not against Fusarium and Rhizopus (Sucher et al., 2009). Caspofungin is administered intravenously (Cancidas™, Merck & Co., Whitehouse Station, New Jersey) at a dose of 70 mg on the first day and 50 mg on the following days (Reddy et al., 1982). Micafungin (Mycamine™, Astellas Ireland, Killorglin, Ireland) is also administered intravenously at a dose of 100 to 150 mg/day (Müller et al., 2013).

Topical caspofungin at a concentration 1.5 to 5 mg/ml was as effective as AMB in the treatment of corneal ulcer by C. albicans in an animal model (Goldblum et al., 2005). Two other studies involving topical micafungin 1 mg/ml found an efficacy comparable to FCZ in the treatment of keratitis by C. albicans and C. parapsilosis (Matsumoto et al., 2005).

All three echinocandins show limited penetration into the aqueous and vitreous humors of laboratory animals after systemic administration, with either undetectable or low concentrations relative to those in plasma (Groll et al., 2001a; Groll et al., 2001b). However, micafungin concentrations specifically in the retina and choroid of the eyes of rabbits range from 0.75 to 15.97 µg/ml and are comparable with the concentrations in plasma (Suzuki et al., 2008). Potentially subtherapeutic vitreal penetration of caspofungin has been associated with treatment failure in C. albicans endophthalmitis (Gauthier et al., 2005). Low concentrations of caspofungin in the aqueous humor of a human endophthalmitis patient is reported (Spriet et al., 2009). Similarly, low micafungin concentrations in the aqueous and vitreous of a C. albicans endophthalmitis patient (0.001% of the simultaneous concentration in plasma) were associated with clinical failure (Mochizuki et al., 2011), and the drug was also
ineffective in a patient with endophthalmitis caused by *C. tropicalis*, despite severe inflammation and a MIC of 0.03 µg/ml (Mochizuki et al., 2012).

In disseminated candidiasis and *Candida* meningitis models in rabbit micafungin was found to be effective when doses higher than those used clinically, were being administered(Petraitiene et al., 2008; Petraitis et al., 2002). A single case report of possible *Candida* endophthalmitis in a patient who appeared to have mild vitritis noted success with caspofungin therapy (Sarria et al., 2005) but others have documented failure with caspofungin and found undetectable levels in the vitreous humor (Gauthier et al., 2005).

Micafungin is a water soluble echinocandin antifungal agent. It can block the formation of biofilms of *Candida* species(Venditti, 2009) but has poor penetration into the vitreous after a single intravenous injection in animal models (Suzuki et al., 2008). Micafungin levels were found to be very low in the vitreous and aqueous humor of seven patients with fungal disease who received intravenous injections of 150-300 mg (mean±SD: 0.08±0.12 µg/ml and 0.10±0.07 µg/ml, in aqueous and vitreous, respectively)(Mochizuki et al., 2013). Mochizuki et al. (2013) recommend that intravenous micafungin be considered only for patients with mild endogenous fungal endophthalmitis (isolated chorioretinitis without vitreous extension) without vitrectomy. Systemic administration of micafungin has good corneal penetration. Because the level of the micafungin in the aqueous and vitreous humors is not high, micafungin may be combined with an intravitreal antifungal agent with vitrectomy for the treatment of severe endogenous fungal endophthalmitis. However Pea (2013) advocate that *Candida* endophthalmitis may benefit by the treatment with a systemic antifungal agent with high penetration in the ocular compartment, as FCZ, VCZ or liposomal AMB.

5 Antifungal Market

The total value of the world market for antifungals (topical as well as systemic) was worth approximately $6 billion, as per reports in 2004. The systemic treatment was supposed to have 60% share of the total revenues, and the remaining 40% (i.e. $2.5 billion) for topical (OTC and prescription) antifungal drugs (Visiongain Report, 2004). Of the systemic antifungal market, azoles (particularly triazoles) were estimated to have 52% share (Maertens, 2004). North America leads the global ophthalmic drugs market, followed by Europe, owing to the increasing support from the government, technological advances, and presence
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of major industry participants. Asia-Pacific is a key market for ophthalmic drugs due to growing patient pool, increase in healthcare expenditure, and infrastructure in emerging economies such as China and India. As per WHO estimates in 2010, in the next nine years, the number of blind people aged 50 years and above will grow in these regions, thereby increasing the demand for ophthalmic drugs. On the other hand, in developed regions such as North America and Western Europe, rising efforts towards prevention of blindness among the aging population has emerged as a key driver for the market. On the basis of treatment drugs, the ophthalmic drugs market is segmented into retinal drugs, dry eye drugs, anti-glaucoma drugs, and ophthalmic inflammatory/anti-allergy/infective drugs (http://www.transparencymarketresearch.com/pressrelease/ophthalmic-drugs-market.htm).

6. Future perspective

Fungistatic nature of available drug options, poor intraocular penetration of topical and systemic antifungals, drug resistance, and non-availability of an effective broad spectrum antifungal agent are main hurdles in ocular antifungal treatment (Anutarapongpan and O’Brien, 2014; Silva et al., 2015). The limited range of antifungal eye drops available for treatment and the lack of favorable outcomes with existing antifungal eyedrops are major hurdles to successful treatment of fungal keratitis; hence, alternatives are urgently needed (Neoh et al., 2014). To increase the antifungal eye drop armamentarium it is important to develop suitable topical products particularly for agents exhibiting broad spectrum activity (Neoh et al., 2014). The topical route is the most frequent and preferred way to deliver drugs to the eye. Unfortunately, the very low ocular drug bioavailability (mostly 1 % or even less) associated with this modality of administration, makes the efficient treatment of several ocular diseases a significant challenge. It has been shown by various studies that specific nanocarriers can interact with the ocular mucosa, thereby increasing the retention time of the associated drug onto the eye, as well as its permeability across the corneal and conjunctival epithelium (Reimondez-Troitino et al., 2015). Hence, development of nanoformulations of azole antifungals, KTZ and FCZ for ocular drug delivery, was taken up by us for better therapeutics.

7. Conclusions

Invariably antifungal agents present significant challenges related to their delivery such as lipophilicity and larger molecular weight to hamper their ocular penetrability
Thus a need is felt for fabricating suitable delivery system for efficient administration of antifungals as eye drops to enhance their levels in the target ocular tissue. Repackaging of these agents will reduce the frequency of administration of drug which is otherwise aggressive. Latter will improve patient compliance and reduce the overall health cost of therapy even if the cost per dose increases due to employment of a novel delivery vehicle.
NANOTHERAPY FOR POSTERIOR EYE DISEASES

1. Introduction
Diseases affecting posterior eye segment are presently increasing at an alarming rate. These include age-related macular degeneration (AMD), diabetic macular edema, viral retinitis, proliferative vitreoretinopathy, posterior uveitis, retinal vascular occlusions, choroid neovascularization (CNV), and diabetic retinopathy, to name a few. Most of these diseases may invariably lead to permanent vision loss if left untreated (Janoria et al., 2007). Of the total debilitating ocular diseases, 55% are posterior segment diseases; while ophthalmic pharmaceutical sales (in the 2007), accounting for the posterior segment ailments was only 5% of the total sale of ocular products. The most common, present day treatment option, for posterior segment ocular disorders is surgery. However, with a better understanding of anatomy of the eye, pathophysiology of these posterior eye diseases and advancement in ocular delivery systems and techniques, several effective novel drug therapies are now being offered as the viable alternatives (Edelhauser et al., 2010).

Treatment of these diseases, requires a direct and local application of the agent to the posterior eye segment at a therapeutic concentration, because delivery of exogenous molecules to the intraocular tissues including the retina is significantly limited (Kompella and Lee, 1999) especially via the topical and systemic routes. Eye is a highly protected organ with several anatomical and physiological barriers in place viz. the cornea and conjunctiva, the blood-aqueous barrier, and the blood-retinal barrier. However, use of nanostructured delivery systems have been shown to defy these barriers and target internal eye tissues, including retina, even following topical application. Present review endeavors to include a variety of such studies in which nanocarrier systems have been developed to overwhelm limited bioactivity and bioavailability of therapeutics to retina and other posterior eye tissues. Although most of these studies are in a preclinical stage but the excitement associated with the promise, such an option holds, makes it highly appropriate to review these studies and explore the plethora of possibilities offered therein.

2. Routes for posterior delivery
The commonly available routes (Fig. 2) to target posterior segment of the eye are-topical, systemic, intravitreal, and periocular, and are discussed below in brief. Table 1 highlights the various aspects of these routes along with advantages and disadvantages associated with each route.
2.1 Topical delivery
Topical delivery is a relatively easy and a less risky method of drug administration. However, delivery to the posterior segment via this route is considered inefficient and unsuccessful, as <5% of the topically applied dose enters the eye and a fraction of it (0.001%) is expected to reach the posterior segment (Sigurdsson et al., 2007). This is attributed to a variety of reasons: (i) limited volume of administration (30 µl); (ii) fast clearance from ocular surface; (iii) metabolism of the active by tear enzymes; (iv) non-productive uptake into systemic circulation via highly vascularized conjunctiva, choroid, uveal tract and inner retina (Ahmed and Patton, 1985; Geroski and Edelhauser, 2000; Hughes et al., 2005); (v) anterior membrane barriers (cornea, conjunctiva, and sclera); (vi) aqueous humor outflow; (vii) long diffusional path (Kato et al., 2004); and (viii) acellular nature of the vitreous which may negatively impact the pharmacokinetics and distribution of topically applied drugs (Maurice, 2001).

2.2 Intravitreal delivery
Intravitreal administration involves direct administration of drug solution/suspension into vitreous humor via pars plana using a 30 G needle (Sarao et al., 2014). In contrast to the topical and systemic routes, intravitreal injection makes high concentrations of drug locally available to the internal eye tissue including the choroid and the retina. Inoue et al. (Inoue et al., 2004) compared sub-Tenon’s and intravitreal injection of triamcinolone acetonide and found that the concentration of triamcinolone acetonide available in the vitreous humor was more when applied intravitreally. Similarly, intravitreal administration of Macugen® (pegaptanib sodium; Pfizer) and Lucentis® (ranibizumab; Genentech/Novartis), the vascular endothelial growth factor (VEGF) inhibitors is highly successful for the control of AMD.

Agents with molecular weight less than 500 Da when applied intravitreally, however, tend to be drained off from the site of application with a half-life of less than 3 days, indicating a need for repetitive injections. Howsoever, the period requiring a repeat dose may extend from a few days to several months for macromolecular antibodies. For example the mean number of injections of bevacizumab (Avastin®, Roche) required to be administered per year for the treatment of AMD is three. On the other hand, the recommended dosing frequency of Ranibizumab (Lucentis®) is once a month (0.5 mg; 50 µl) for a minimum of nine months (Rosenfeld et al., 2006) while Macugen® needs to be injected intravitreally at 6 week intervals for at least one year (Kitagawa and Yuzawa, 2013).
Nevertheless repetitive intravitreal injections, even if spaced widely are invariably associated with complications, such as vitreous hemorrhage, retinal detachment, cataract and endophthalmitis. Rate of endophthalmitis and retinal detachment being observed with intravitreal injection is 0.2% and 0.05% respectively (Edelhauser et al., 2010). Moreover, patient compliance is lower with such regimens because of the painful and invasive procedures requiring hospitalization and specially trained physician for administration adding to the cost, in addition to the high cost of the medicine per se.

2.3 Periocular delivery

Periocular route refers to administration of drug to the region surrounding the eye and includes subconjunctival, peribulbar, posterior juxtascleral, sub-tenon’s and retrobulbar injections. Permeation of radiolabeled mannitol following subconjunctival injection to rabbits indicated direct penetration through sclera as the primary pathway for delivery of materials to the posterior segment, followed by systemic recirculation and a minor transcorneal uptake (Cheruvu et al., 2008; Lee and Robinson, 2001; Ranta et al., 2010) (Fig. 2). Periocular route though not as efficient as the intravitreal route offers an advantage of lesser invasiveness. A better retinal and vitreal drug bioavailability (about 0.01–0.1%) is achieved via this route in comparison to the topical route of application (about 0.001% or less) (Kim et al., 2004; Urtti et al., 1990). Repetitive periocular administration under local anesthesia, is possible without direct interference with the vision. Volumes as high as 500–5000µl of drug solution can be administered via periocular route in humans (Cheruvu et al., 2003) versus only 50–100µl being administrable, via intravitreal route.

Evidence suggests that ocular tissue concentrations are higher following periocular routes of administration compared to intravenous, topical, and oral administrations (Kalsi et al., 1991; Maurice, 2001).

2.4 Systemic delivery

Availability of drug in the posterior eye segment following systemic administration as tablets, capsules or intravenous injections is limited by the presence of the blood retinal barrier (BRB), which is selectively permeable to highly lipophilic molecules. Lipid-soluble drugs such as chloramphenicol and minocycline penetrate the BRB, while aminoglycosides (amikacin) and β-lactams (cefazolin) being hydrophilic even though proposed for, use in the treatment of endophthalmitis, do not reach the
vitreous in sufficient concentrations following systemic administration (Boddu and Nesamony, 2013). 

Low bioavailability (1%-2%) of the drug at the BRB (Boddu and Nesamony, 2013) demands frequent administration at high doses, resulting in serious systemic side effects (Hughes et al., 2005).

![Fig. 2 Pathways for distribution of drug to the retinal tissue of the eye following different delivery routes. Italicization indicates non-productive drug losses.](image)

3. **Barriers to posterior delivery**

Different anatomical and physiological barriers (Table 1), which constitute the protective machinery of the ocular tissues against exogenous materials, prevent the permeation of therapeutic agents to the retina.

Even though the intravitreal injection applies the drug directly to the vitreous yet it does encounter the neural retina (Fig. 3) and the vitreal hydrogel, thatform diffusion barriers to the availability of drug at the retina.

Neural retina (Fig. 3) is the layer that separates the underlying pigmented retinal layer from the vitreous humor. It is a multilayered structure, with the inner (ILM) and outer limiting membranes enveloping inter-photoreceptors, forming significant barrier to passage of materials. These layers, rich in glycosaminoglycans, bind to cationic molecules and limit their transport through the retina. Pitkanen et al. (2003) found that the cationic charge is a more important limiting factor than the molecular size for transport across the neural retinal barrier. Fluorescein isothiocyanate-dextran (FITC-dextran) of mean molecular weight 20,000 permeated well through the retina with
87% of retinal pigmented epithelium cells showing fluorescence, while the permeation of positively charged fluorescein isothiocyanate labeled poly-L-lysine (FITC-PLL) of the same molecular weight was practically blocked (3% of retinal pigmented epithelium cells positive). Permeation of an even larger molecular weight (20,000,000) FITC-dextran molecule, through the neural retina, was still better than FITC-PLL. This effect was attributed to the binding of polycations including the positively charged nanocarrier systems, to the negative biomacromolecules of neural retina, limiting their passage across it (Kwan et al., 2006). Howsoever, ILM comprising of a meshwork of pores ranging in size from 10-25nm, acts as a barrier to the movement of macromolecules from the vitreous to the retina and it has been reported by Kamei et al. (1999) that a even 70kDa tissue plasminogen activator could not diffuse through ILM on intravitreal injection.

The vitreous body is mainly composed of water (98%–99% w/w) with few solid components viz. collagen and glycosaminoglycans. Latter, although present in small amounts, are organized to form and maintain a stable gel such that it impedes the free diffusion of molecules, particularly high-molecular weight compounds or suspended solids through the vitreous (Le Goff and Bishop, 2008; Mains and Wilson, 2013). Natural vitreous aging (known as syneresis), any previous vitreoretinal surgery, and multiple injection procedures may influence the distribution characteristics and kinetics (Berezovsky et al., 2011) of drugs administered intravitreally.

In addition to the above considerations, BRB, primarily composed of tight junctions of retinal endothelial blood vessels and retinal pigment epithelium (RPE), acts as a significant barrier to drug absorption into the retina and vitreous via the systemic route (Kiernan and Lim, 2010). In a study conducted in monkeys, horseradish peroxidase (44 kDa) was retained and could not cross the tight junctions of the RPE (Toris and Pederson, 1984).

Factors like binding of the drug to the tissue proteins or melanin, active transport processes, and metabolism also tend to affect the permeability of molecules across RPE (Kennedy and Mangini, 2002). Ocular absorption of drugs after systemic administration (Cunha-Vaz, 2004) or through transscleral routes (Pitkanen et al., 2005) is thus restricted by the RPE. It also limits the permeation of drugs from the choroid to the retina.

Pitkanen et al. (2005) found RPE to be the rate-limiting permeation barrier to the hydrophilic molecules and macromolecules, such as oligonucleotides and proteins, via
the transscleral route. The permeation lag time through the RPE increases with the increase in molecular weight as the former is inversely proportional to the diffusion coefficient. Permeability of the fluorescent FITC-dextran probes through RPE-choroid decreased significantly with the increasing size of the probe. RPE-choroid was found to be 35 times more permeable to carboxyfluorescein (376 Da) than to FITC-dextran 80 kDa. Further to this, the permeation of lipophilic β-blockers was significantly higher than that of hydrophilic atenolol (8 times) and carboxyfluorescein (20 times). Bovine RPE-choroid was 10 to 100 times less permeable to hydrophilic compounds and macromolecules as compared to the sclera. The permeability of lipophilic molecules through the RPE-choroid was however similar to that observed for the sclera. Further, small, lipophilic (Pitkanen et al., 2005; Ranta and Urtti, 2006) and/or cationic molecules bind to melanin in the RPE which may also compromise their permeation across it (Edelhauser et al., 2010).

Fig. 3 Schematic representation of different layers of retina and the available transport options across the retinal pigmented epithelium.
<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Pathway</th>
<th>Barriers involved</th>
<th>Elimination</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Intravitreal             | Drug is delivered directly to the vitreous chamber | Diffusion through the vitreous chamber; neural retina; blood retinal barrier (BRB) | Movement to aqueous chamber and retina | a) Local and direct delivery  
b) High therapeutic concentration | a) Repeat injection  
b) Rapid elimination  
c) Serious side effects may occur on repetitive injections especially retinal detachment, cataract, vitreous hemorrhage and endophthalmitis |
| Periocular               | Majorly via the transscleral pathway | Sclera; BRB; choroid | Conjunctival and choroidal blood and lymphatic flow. Losses from the periocular space, BRB, choroidal circulation and binding of drugs to | a) Least painful  
b) Most efficient route  
c) High therapeutic drug levels | a) Rapid clearance  
b) Systemic side effects  
c) Tissue hemorrhage |
| Systemic | Choroid, conjunctiva and scleral pathway | tissue proteins; efflux transporters. | BRB; choroid; efflux transporters | Hepatic clearance, conjunctival and choroid capillaries | Better patient compliant | a) Low bioavailability  
b) High doses required  
c) Systemic side effects |
|----------|----------------------------------------|---------------------------------|-------------------------------|-------------------------------------------------|--------------------------|-----------------------------|
| Topical  | Corneal, conjunctival and scleral pathway | Cornea, BRB | Tear wash out, nasolacrimal drainage, choroid and conjunctival blood flow | a) High patient compliance  
b) Less systemic side effects | a) Small retention time  
b) Blurring of vision  
c) Precorneal drug losses  
d) Drainage through the nasolacrimal duct  
e) Irritation  
f) Low bioavailability |
The high permeability of sclera to hydrophilic molecules is comparable to corneal stroma (Geroski and Edelhauser, 2000; Kuno and Fujii, 2011). The molecules are expected to diffuse through the interfibrillar aqueous media of the gel-like proteoglycans. Transscleral route may be suitable for delivery of biotech-drugs to the retina and vitreous, if appropriate delivery systems are developed (Del Amo and Urtti, 2008). Thus, sclera, with its larger surface area (about 95% of total eye surface), hypocellular nature, ease of access (Ambati and Adamis, 2002) and high permeability characteristics is shown to be a promising route for delivery of both small (Ahmed and Patton, 1985) and high (Ambati et al., 2000) molecular weight drugs to the retina.

The permeability of drug molecules across the sclera is inversely proportional to the molecular weight as well as lipophilicity of drug molecules (Berezovsky et al., 2011; Prausnitz and Noonan, 1998; Thakur et al., 2011). Berezovsky et al. (2011) observed that the 40kDa dextran reached a higher peak concentration (2.3 times) than the 70kDa dextran by sub-Tenon’s injection and also underwent a faster elimination than its counterpart. However, at the same time, few researchers report that high-molecular weight compounds (e.g., FITC-dextran, 150 kDa) that cannot reach the chorioretinal tissues after intravitreous administration because of the barrier provided by the inner limiting membrane (ILM) can reach retina by transscleral injection or upon application of an episcleral implant for site-specific treatment (Ambati et al., 2000; Kato et al., 2004).

Choroid, especially Bruch’s membrane, is also a significant barrier to permeation of macromolecules (Moore and Clover, 2001). Furthermore, the pigmented nature of choroid and its affinity for lipophilic solutes, also limits the retinal availability of these molecules (e.g., celecoxib) (Cheruvu et al., 2008) administered by transscleral route. It may however be noted that major retinal availability of drugs administered systemically is via the choroid tissue which is a highly vascularized part of the eye. Howsoever, efflux transporters such as P-glycoprotein (Pgp) and multidrug resistance associated proteins (MRP) found in both apical and basolateral cell membranes of human RPE may limit the permeation from choroid to retina and vitreous chamber (Boddu and Nesamony, 2013; Hughes et al., 2005; Kennedy and Mangini, 2002). These proteins belong to ATP binding cassette (ABC) transporter family since these
efflux pumps use energy from ATP hydrolysis. These transporters mainly extrude molecules out of the cytoplasm. Furthermore, drug losses via the conjunctival and episcleral blood and lymphatic flow are the other limiting factors to posterior segment drug distribution especially of small molecules upon subconjunctival administration (Ghate et al., 2007), whereas anatomic barriers and choroidal blood flow are considered less important (Chan et al., 2010; Liu et al., 2010). However, the elimination of proteins is 1–2 orders of magnitude slower (Kim et al., 2008; Ranta et al., 2010). This was proven in experiments with post-mortem rabbits wherein blocked blood and lymphatic flow from the subconjunctival site resulted in a significantly improved delivery to the posterior segment (Chan et al., 2010; Kim et al., 2008).

The activity of influx transporters (neutral and cationic amino acid transporter, folate receptor-α and sodium dependent multivitamin transporter) on the other hand favorably influence the drug uptake (Fig. 3). Expression of these transporters in human RPE cells may, in turn, be regulated by certain physiological states such as hypoxia (Vadlapatla et al., 2013) which results in an enhanced uptake relative to normoxia in retinal cell lines. An elaborate knowledge of these transporters may thus help to develop suitable approaches for effective ocular drug delivery.

It may thus be summed up that the conventional ocular formulations invariably cannot overcome the variety of barriers to ocular delivery. It is in this context that the use of nanocolloidal drug delivery systems, such as nanoparticles, nanoemulsions and liposomes, are being promoted as a possible way to enhance the bioavailability of administered drugs and overwhelm the existing barriers for effective retinal delivery.

4. Nanostructured Drug Delivery Systems

Owing to the invasive nature of available route of administration and need for repetitive administration of drug agent for the treatment of posterior eye diseases, it is important to design such formulations which can maintain requisite therapeutic drug concentration in the posterior segment of the eye for prolonged periods. Latter will minimize the number of local injections or repetitive systemic, oral or parenteral administrations required to achieve an effect. Use of nanoparticulate systems—liposomes, nanoparticles and nanoemulsions, with size <1000nm have been explored as an appropriate alternative to conventional options (Fig. 4). These systems are purported and have been experimentally confirmed in an array of studies, to bypass
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various ocular barriers and transport drugs to the posterior segment of the eye (Kakkar and Kaur, 2011; Pepiæ et al., 2012).

Further, nano size avoids irritation which is generally an issue with ocular suspensions or microsized (>10µ) particle considering the highly sensitive nature of ocular tissue (Hecht, 2001; Nagarwal et al., 2009).

The incorporation of drugs into nanocarrier systems would lead to improved biopharmaceutical properties including solubility, stability, permeability, retention at the site of application and release of drug over an extended period. This is complemented with the possibility of a simple and inexpensive sterile production like aseptic filtration (particle size usually less than 100 nm) and also autoclavability (Abrishami et al., 2009; Nagarwal et al., 2009; Pepiæ et al., 2012).

These systems are equally effective for administration of both the lipophilic and hydrophilic drugs. Since lipophilic drugs are difficult to formulate as drops or injection for ocular delivery and hydrophilic drugs show poor permeability across ocular tissues, hence encapsulating them in nanocarrier systems provides a productive means of administering these drugs.

Furthermore, most of these systems are aqueous dispersions and their direct application in the form of eye drops or injections is easily possible (Abrishami et al., 2009). Development of these systems as topical eye drops with a potential to carry the drug across a series of barriers and ultimately deliver their cargo in the posterior eye segment is a promising option. Eye drops are a patient preferred ocular forms due to their ease of administration which is non-invasive and pain-free in addition to low cost.

The nanocarrier systems can act as a depot which provide controlled and prolonged release of drug at the desired site. This results in a reduced frequency of instillations; and the latter is a very important concern both for the invasive treatment options (Eljarrat-Binstock et al., 2010) and in case of chronic ailments requiring frequent applications and prolonged therapy, invariably compromising compliance. Kompella et al. (2003) reported that the retinal level of budesonide were 2 and 9 times higher with nanoparticles than the budesonide solution-treated groups at the end of day 3 and 7, respectively, upon subconjunctival injection. Apart from this, the nanocolloidal systems also allow for a prolonged precorneal retention, protection to drug, and improved ocular bioavailability upon topical application. Since most of the agents
being used in treatment of ocular diseases were not specifically designed for ocular tissues hence their entrapment in nanocarrier systems provides an effective means of permeation and delivery to the internal eye tissues. Howsoever, the structure of the tissue may impact the rate and extent of penetration of nanoparticles through the vitreous to reach the ILM. In addition, influence of flow systems operating within the eye, age-related structural changes, and various modifications associated with tissue inflammation may potentially influence the free movement of nanoparticulate systems. For effective penetration to the vitreous, the physical properties, namely, the size and charge, of the nanoparticles are among the key attributes which can influence the performance of a formulation and should be taken into account when designing an effective ocular drug delivery system (Fig. 4).

Nagarwal et al. (2009) indicated that uptake of PLGA particles in rabbit conjunctival epithelial cells was higher for 100nm particles than larger (800nm and 1000 nm) particles; also 100 nm particles were able to cross the cornea efficiently. Nanocarriers because of their size and ability to adhere to the tissue surface are better retained in the cul-de-sac of the eye (Fig. 5). Furthermore, their interaction with the glycoproteins of the cornea and conjunctiva can form a precorneal depot resulting in prolonged release of the encapsulated drug. Laffleur and Bernkop-Schnürch (2013), attributed the enhanced retention of nanocarriers to their entanglement in the mucin layer (Fig. 5) covering the corneal epithelium due to their small size. However, Amrite et al. (2008) found that periocular administration of very small particles of 20nm size were cleared by blood and lymphatics such that they were lost into systemic circulation without achieving any ocular effect and significant permeation across sclera-choroid-RPE.

Peeters et al. (2005) found that cationic liposomes showed aggregation in vitreous while anionic liposomes were uniformly distributed due to reduced binding to biomacromolecules. Similar observations were made by Kim et al. (2009), who noted aggregation of cationic human serum albumin nanoparticles on intravitreal administration. Likewise, Koo et al. (2012) reported that the strongly charged cationic polyethyleneimine nanoparticles showed strong interaction with the anionic vitreal collagen fibrils bridged by glycosaminoglycan filaments. Anionic nanoparticles on the other hand showed a superior penetrability across all the layers of retina.

Recently, these nanocarrier systems have also been explored extensively for their application in gene delivery to the posterior eye segment. Gene delivery has evolved...
as a treatment option for several posterior eye diseases viz. AMD and retinal neovascularization. Since, most genetic material is degraded in the cellular environment hence nano-encapsulation is intended to (i) provide protection to the entrapped genes or genetic material; (ii) carry them across various biological barriers, and (iii) help in their transfection (Conley and Naash, 2010). Nanoparticles with particle size less than 25nm have the capability to pass through the pores of nuclear membrane, thus providing efficient transfection (Liu et al., 2003).

Subsequent passages discuss application of various nanocarrier systems for effective posterior eye delivery, illustrated with suitable examples from literature.

4.1 Liposomes

Liposomes are composed of a lipid bilayer formed from phospholipids and cholesterol surrounding an aqueous compartment, which allows encapsulation and protection of drug. They facilitate slow release of encapsulated drugs without altering its intrinsic characteristics. Furthermore, they are non-toxic and biocompatible and have the ability to effectively encapsulate the hydrophilic and lipophilic drugs.

Table 2 demonstrates the wide applicability of liposomal systems for posterior eye delivery, however some illustrative examples are discussed below.

Intravitreally administered liposomal systems not only increase drug $t_{1/2}$ (Tremblay et al., 1985) but also minimize any intraocular side effects associated with the use of entrapped agents (Fishman et al., 1986). Radiolabelled oligonucleotide ([33P]pdT16) liposomes showed a significantly longer retention than the free oligonucleotide solution in the vitreous chamber. This was attributed to the decrease in the degradation (Bochot et al., 2002) of oligonucleotide entrapped within the liposomes. After 24 h of administration, the residual concentration of pdT16 within the vitreous was 9.3-fold higher when administered as a liposomal suspension in comparison to the free pdT16 solution.
Fig. 4 Flowchart signifying issues with conventional ocular dosage forms, paving way to alternative nanocarrier systems coupled with topical route of administration. Figure also discusses the factors to be considered and issues related to these nanoscale delivery options.
Fig. 5 Schematic representation of proposed pathways to posterior eye segment followed by administration of nanocarrier systems: (1) through intravitreal injection; (2) via blood retinal barrier following systemic delivery; 3) transscleral route following subconjunctival or periocular delivery; 4) lateral non-corneal diffusion pathways following topical administration (5) corneal pathway following topical application. ❄ Slow drug release from the nanostructured carrier system acting as a depot in the vitreous and retinal cells.

*,#,¥ and ₤ indicate uptake mechanisms of nanocarrier systems upon topical application. *- Nanocarriers retained in the mucin layer and/or being mucoadhesive achieve increased precorneal residence time; #- Adhesion to corneal epithelium; ¥- Nanocarrier in the vitreous humor; ₤- Nanocarriers approaching retina.
A single intravitreal injection of liposome-encapsulated cidofovir prevented *Herpes simplex* virus retinitis for 4 months (Kuppersmann et al., 1996) in experimental model of HSV-1 retinitis. This demonstrates that a sustained release of the drug in the posterior segment is possible from a liposomal carrier and it could be a viable option for treatment of retinal diseases.

The presence of intact liposomes in retina after topical delivery, on the other hand, was indicated by Hironaka et al. (2009, 2011). They proposed that the liposomes travelled majorly through the corneal and conjunctival route involving the iris and ciliary body. Such a transport mechanism was attributed to the rigidity and nano size of the liposomes. It was found that fluorescent liposomes prepared using L-α-distearoylphosphatidylcholine showed higher fluorescence emission in the retina than those prepared using egg phosphatidylcholine, and that the former were more rigid than the latter. Rigidity maintains the stability of the carrier system in the biological environments such as tear film and ocular mucosa.

Tremblay et al. (1985) found that the ocular toxicity was significantly reduced when liposome-intercalated amphotericin B was intravitreally injected as compared to commercial amphotericin B (for intravenous use which contains sodium desoxycholate as a solubilizing agent).

Davis et al. (2014) demonstrated that liposomes comprising of annexin A5 showed a significantly enhanced uptake and transcytosis across corneal epithelial barriers, after topical administration, and delivered appreciable amount of Avastin (bevacizumab) to the posterior segment of the eye including retina after topical application. The authors conclude that the anionic phospholipid binding protein, annexin A5, ensures efficient endocytosis and hence an enhanced uptake of lipidic drug delivery systems across biological barriers. Likewise, pDNA loaded liposomes showed high encapsulation efficiency and good cellular uptake ability in human retinal pigment epithelial cells (ARPE-19 cells). Transferrin modified liposomes ensure binding to specific receptor on the RPE cells leading to highly improved gene delivery to the posterior segment of eye by instillation as eye drops (Takashima et al., 2012).

Foscarnet encapsulated liposomes show slow release extending beyond 72h in comparison to the free drug. The relative bioavailability of the liposomal formulation was about 2.5 and 5 times that of the free drug solution in vitreous and retina, respectively (Claro et al., 2009).
Diaz-Llopis et al. (1992) also demonstrated that detectable quantities of ganciclovir were observed in the intraocular tissues, even 14 days after administration of a single intravitreal injection of liposomally-entrapped ganciclovir without any observable damage to the retinal structures. Complete remission of the cytomegalovirus (CMV) retinitis was observed in the AIDS patients after the administration of third injection (one per week) and no relapse was observed during the 2-4 month follow-up of the patients. Further to it, the required frequency of administration was substantially decreased for the intravitreal liposomal formulation.

Despite these advantages that make liposomes a potentially useful system for ocular drug delivery, their utility is limited due to the short shelf life, limited drug-loading and difficulty in sterilization (Sahoo et al., 2008).

Howsoever, the liposomal technology has been explored extensively and some such products for the treatment of retinal diseases are already in market. For example, Visudyne® (Novartis Pharmaceuticals, USA), a liposomal formulation incorporating verteporfin is used for the treatment of choroidal neovascularization due to AMD, pathologic myopia, or ocular histoplasmosis. It is the first drug approved by the FDA in 2000, for the treatment of wet age-related macular degeneration. After 15 minutes of intravenous infusion of Visudyne®, a non-thermal red laser light with a wavelength of 693 nm laser is applied to the retina to activate verteporfin that causes local damage to endothelium, resulting in the blockage of targeted vessels (Ruiz-Moreno et al., 2006). It may however be noted that the photodynamic therapy on its own may increase local production of VEGF resulting in a potential reappearance of the choroidal neovessels. Visudyne® was found to be insufficient in some such cases and the patients thus required repeated treatments (Mishra et al., 2011; Moshfeghi and Peyman, 2001).

Photrex® (Miravant Pharmaceuticals) containing rostaporfin is another liposomal light activated formulation indicated for the treatment of AMD. Photrex® has completed Phase III clinical studies and is awaiting FDA approval (Rivers and Hughes, 2013). The frequency of the required treatments is significantly lower than that of Visudyne®.

It may be noted that both the liposomal formulations are for systemic (parenteral) administration and not for direct/local administration to the eye. In our opinion it will
be more fructuous if the efforts are made to offer non-invasive ocular drops using liposomal systems.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of administration</th>
<th>Observation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>Intravitreal injection</td>
<td>The half life of liposomes was longer as compared to free drug in healthy and endophthalmitis eye</td>
<td>(Zeng et al., 1993)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Intravitreal injection</td>
<td>Lower toxicity than amphotericin B deoxycholate solution and amphotericin B lipid complex Reduced toxicity and need for repetitive administration in comparison to free drug, for the control of chronic disorders requiring persistent low concentration of amphotericin B</td>
<td>(Cannon et al., 2003)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Intravitreal injection</td>
<td>Five times higher vitreous concentration in comparison to the free drug treated eyes, in 42 days</td>
<td>(Abrishami et al., 2009a)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Topical</td>
<td>Liposomes comprising of annexin A5 significantly enhanced uptake and transcytosis of liposomes across corneal epithelial barriers after topical administration and delivered appreciable amount of entrapped bevacizumab to the posterior of the eye including retina after topical application.</td>
<td>(Davis et al., 2014)</td>
</tr>
<tr>
<td>Cidofovir</td>
<td>Intravitreal injection</td>
<td>Showed a protective effect for 8 months in Herpes simplex model Liposomes showed prolonged (up to 4 months) antiviral effect in the experimental model of HSV-1 retinitis.</td>
<td>(Besen et al., 1995)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Intravitreal injection</td>
<td>Absence of significant retinal toxicity</td>
<td>(Wiechens et al., 1999)</td>
</tr>
<tr>
<td>Coumarin-6</td>
<td>Topical</td>
<td>Surface modified liposomes, entrapping poly L-lysine, size dependently, delivered coumarin-6 to retina. Interaction of</td>
<td>(Sasaki et al., 2013)</td>
</tr>
<tr>
<td>Drug</td>
<td>Route of Administration</td>
<td>Effect Description</td>
<td>Reference</td>
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<tr>
<td>Cyclosporine</td>
<td>Intravitreal injection</td>
<td>A longer t₁/₂ of 3 days as compared to 6h for free drug was achieved. No retinal toxicity was observed.</td>
<td>(Alghadyan et al., 1988)</td>
</tr>
<tr>
<td></td>
<td>Topical</td>
<td>Cyclosporine loaded liposomes were able to target vitreous humor however, concentration was not significantly different from the corresponding anionic microemulsion (Restasis®).</td>
<td>(Nikoofal-Sahlabadi et al., 2013)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Topical</td>
<td>1.8 times higher delivery efficiency than free drug solution to the retina-choroid.</td>
<td>(Fujisawa et al., 2012)</td>
</tr>
<tr>
<td>Edaravone</td>
<td>Topical</td>
<td>Liposomes significantly inhibited light-induced retinal damage in mice model as compared to free drug.</td>
<td>(Shimazaki et al., 2011)</td>
</tr>
<tr>
<td>FK506</td>
<td>Topical</td>
<td>Significantly higher concentration of FK506 was found when liposomes were used in comparison to the free drug solution in vitreous humor.</td>
<td>(Pleyer et al., 1993)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Intravitreal injection</td>
<td>Reduced retinal toxicity</td>
<td>(Velpandian et al., 2006)</td>
</tr>
<tr>
<td>5-fluorouridine (FUR)</td>
<td>Intravitreal injection</td>
<td>Considerably longer t₁/₂ of 18.17±2.43 h with respect to only 0.82 h for free drug.</td>
<td>(Garcia-Arumi et al., 1997)</td>
</tr>
<tr>
<td>5-fluorouridine 5'monophosphate (FUMP)</td>
<td>Intravitreal injection</td>
<td>Longer t₁/₂ of 124 h after intravitreal administration of 1.0 mg of FUMP in liposomes in comparison to only 4.5 h for free drug.</td>
<td>(Assil et al., 1991)</td>
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</table>

No toxic effects on the retina (Gariano et al., 2010)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foscarnet</td>
<td>Intravitreal injection</td>
<td>The relative bioavailability in vitreous was about 2.5 times that of free drug solution</td>
<td>(Claro et al., 2009)</td>
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<tr>
<td></td>
<td></td>
<td>Increased residence time and stabilization of retinitis with no new lesions while treatment with</td>
<td>(Akula et al., 1994)</td>
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<td></td>
<td>free drug solution resulted in reactivation of CMV retinitis. No ocular tissue damage with</td>
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<td>liposomes.</td>
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<td>Vitreous concentration higher than ID50 (50% inhibitory dose) for Herpes simplex, even on the</td>
<td>(Peyman et al., 1987)</td>
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<td></td>
<td></td>
<td>28th day post administration.</td>
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<td></td>
<td></td>
<td>No retinal toxicity, and therapeutic levels were detectable(4.67±0.39 µg/ml) up to day 14 after</td>
<td>(Diaz-Llopis et al., 1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>injection.</td>
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<tr>
<td>Ganciclovir</td>
<td>Intravitreal injection</td>
<td>Significantly higher vitreous concentration as compared to drug solution.</td>
<td>(Shen and Tu, 2007)</td>
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<tr>
<td></td>
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<td>Increased residence time and stabilization of retinitis with no new lesions while treatment with</td>
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<td>free drug solution resulted in reactivation of CMV retinitis. No ocular tissue damage with</td>
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<td>liposomes.</td>
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<td>Vitreous concentration higher than ID50 (50% inhibitory dose) for Herpes simplex, even on the</td>
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<td>28th day post administration.</td>
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<td></td>
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<td>No retinal toxicity.</td>
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<td></td>
<td></td>
<td>Therapeutic levels were detectable(4.67±0.39 µg/ml) up to day 14 after injection.</td>
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</tr>
<tr>
<td>Gentamicin</td>
<td>Intravitreal injection</td>
<td>A twofold increase in bioavailability as compared to free drug solution</td>
<td>(Fishman et al., 1986)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Intravitreal injection</td>
<td>No retinal toxicity</td>
<td>(Wiechens et al., 1998)</td>
</tr>
<tr>
<td>pDNA (Plasmid DNA)</td>
<td>Topical</td>
<td>pDNA loaded liposomes showed high encapsulation efficiency and good cellular uptake into human</td>
<td>(Takahshima et al., 2012)</td>
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<tr>
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<td>retinal pigment epithelial cells (ARPE-19 cells).</td>
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<tr>
<td>Pigment epithelium</td>
<td>Intravitreal injection</td>
<td>PEDF-loaded liposomes conjugated with peptide ATWLPPR under ultrasonic exposure transmitted</td>
<td>(Li et al., 2010)</td>
</tr>
<tr>
<td>derived factor (PEDF)</td>
<td></td>
<td>PEDF into cytoplasm with high specificity and efficiency to increase CNV inhibitory action of</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>PEDF.</td>
<td></td>
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<tr>
<td>Plasmid DNA</td>
<td>Topical</td>
<td>Efficient transfer of gene to retina could be obtained even after 1 month of administration</td>
<td>(Masuda et al., 1996)</td>
</tr>
<tr>
<td>with β-galactosidase</td>
<td>delivery, intravitreal and</td>
<td>following delivery by all the three routes.</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>subretinal injection</td>
<td></td>
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<tr>
<td>Radiolabelled</td>
<td>Intravitreal injection</td>
<td>The vitreous humor retained &gt;37% of the administered antiviral oligonucleotides</td>
<td>(Bochot et al., 2002)</td>
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<tr>
<td>oligonucleotide</td>
<td></td>
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</tbody>
</table>
Literature Review

<table>
<thead>
<tr>
<th>Literature Source</th>
<th>Drug</th>
<th>Method</th>
<th>Result</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Zhang et al., 2010</td>
<td>Tacrolimus</td>
<td>Intravitreal injection</td>
<td>Vitreous concentration of &gt; 50ng/mL was maintained for 14 days without any observable side effects on retina. Significantly more efficient than free drug in suppressing uveoretinitis.</td>
<td></td>
</tr>
<tr>
<td>Liu et al., 1987</td>
<td>Trifluorothymidine</td>
<td>Intravitreal injection</td>
<td>Prolonged vitreal drug level within the range of ID50 for many strains of Herpes virus and CMV even after 28 days post injection, with no retinal toxicity</td>
<td></td>
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</tbody>
</table>

4.2 Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are polymeric colloidal particles which adsorb, absorb, attach or encapsulate (dissolve or disperse) drug molecule (Birch and Liang, 2007). PNPs have been engineered from various synthetic and natural biocompatible polymers. PNPs derived from natural materials like albumin can serve as an efficient drug delivery system as they are biodegradable, nontoxic and non-immunogenic. Albumin also has a high content of charged amino acids, which permits attachment of positively or negatively charged drugs and oligonucleotides (Sahoo et al., 2008) on it. The most commonly used synthetic polymers are polylactide (PLA) and poly(lactide-co-glycolide) (PLGA), which degrade in vivo to form natural metabolites (lactic and glycolic acids) that are eliminated from the body through the Kreb’s cycle. Their degradation rate can be tailored via changes in co-polymer composition and molecular weight, and a conformation that can provide controlled drug release ranging from months to years (Grizzi et al., 1995; Kranz et al., 2000; Mainil-Varlet et al., 1997). Being biocompatible, biodegradable, non-toxic and non-antigenic, numerous PLGA containing therapeutics are approved by FDA. PNPs offer numerous advantages of controlled ocular drug release as demonstrated below in Table 3 and also in the following illustration (Fig. 5). Fluorescent labeled PNPs were found to be internalized into the retina and remained in the RPE cells for 4 months with no toxic effect following a single intravitreal injection (Bourges et al., 2003). Thus, PNPs can provide a steady and continuous delivery of drugs simultaneously avoiding the requirement for repetitive administration and hence intraocular surgical procedures (Table 3).
PNPs are reported to pass through the retinal layers, accumulating in RPE when injected into the vitreous of rabbits (Bourges et al., 2003; Sakurai et al., 2001). Kim et al. (2009) demonstrated that intravitreally injected anionic human serum albumin-nanoparticles diffused readily through the vitreous and internalized in the RPE. Authors demonstrate promise of the approach for drug carriage to the RPE and choroid. Tamoxifen incorporated into polyethylene glycol (PEG)-coated cyanoacrylate nanoparticles inhibited onset of experimental autoimmune uveoretinitis in an animal model as compared to free drug. The PNPs were retained for 3-9 days, post injection (Kozak et al., 2004).

Zhang et al. (2009) showed that the dexamethasone-PLGA nanoparticles sustained the drug release for at least 50 days in the rabbit eyes, during which relatively constant drug levels were obtained for about 30 days, in the vitreous, with the mean concentration of 3.85 mg/l. Concentration in vitreous was significantly higher than in the plasma, indicating minimum systemic side effects. PNPs improve residence time, while the ability to target drugs to the sight of action leads to a decrease in the required dose (Vandervoort and Ludwig, 2007) and dosing frequency.

They can also achieve cellular delivery either via endocytosis or phagocytosis, providing internalization of encapsulated material (Fig. 6), which may include proteins, DNA, siRNA, lipids and organic/inorganic substances. They additionally provide protection to the molecular integrity of the encapsulated therapeutic agent thereby preventing their rapid in vivo degradation (Janoria et al., 2007).

Subretinal injection of DNA entrapped in nanoparticles showed that nanoparticles remained stable inside the cell leading to a higher and sustained level of gene expression. Further, the nanoparticles were distributed throughout the RPE cell layer (Koirala et al., 2011).
**Fig. 6 Uptake mechanisms of nanoparticulate systems administered to the eye surface.**

*Majorly by Clathrin-mediated endocytosis*

PLGA nanoparticles have been shown to evade the endo-lysosomal formation hence providing protection to the genetic material. Further, these nanoparticles release their payload slowly in the cytoplasm, thus provide sustained therapeutic effect (Fig. 6) (Panyam et al., 2002).

Bejjani et al. (2005) showed effective transfection and protein expression of green fluorescent protein plasmid or red nuclear fluorescent protein plasmid entrapped in PNPs in ARPE-19 human RPE cell line. Li et al. (2006) effectively encapsulated pigment epithelium-derived factor (PEDF peptide) in PLGA nanoparticles which significantly reduced retinal ganglion cell death in mouse retinal ischemia as compared to free PEDF peptide. PNPs were found to be effective for 7 days while free peptide was effective only for 2 days.

Fluorescent nanoparticles incorporated in a hydrogel applied on to cornea and sclera when subjected to iontophoresis (1.5mA for 5 min) (Eljarrat-Binstock et al., 2008) ensured posterior eye targeting for upto 12h. The positively charged particles showed better florescence in the inner ocular tissue than negatively charged particles. This was attributed to negative charge of corneal and conjunctival mucosa which leads to
increase in concentration and residence time of drug incorporated into positively charged particles.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of administration</th>
<th>Observation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TGF-b2 PS-DN</td>
<td>Intravitreal injection</td>
<td>PNP shows homogenous retinal distribution within 24 h of intravitreal administration.</td>
<td>(Gomes dos Santos et al., 2006)</td>
</tr>
<tr>
<td>Basic fibroblast growth factor</td>
<td>Intravitreal injection</td>
<td>PNP prevents photoreceptor degeneration by inhibiting apoptosis in rat retina. Rhodamine labeled PNP were seen for 8 weeks in outer nuclear layer.</td>
<td>(Sakai et al., 2007)</td>
</tr>
<tr>
<td>Betamethasone phosphate</td>
<td>Intravenous injection</td>
<td>PLA-NPs showed significant effect in experimental autoimmune uveoretinitis and were observed up to 7 days post-injection.</td>
<td>(Sakai et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stealth PLA-NPs showed significant effect in treatment of autoimmune uveoretinitis in rat model which was sustained for 2 weeks. Rhodamine labeled NPs were seen localized in retina and choroid.</td>
<td>(Sakai et al., 2011)</td>
</tr>
<tr>
<td>Topical</td>
<td>Topical</td>
<td>Betamethasone phosphate loaded mucoadhesive chitosan-sodium alginate NPs showed prolonged release and significantly high concentration in vitreous humor (68% of the drug over 6 h, and then decreased to 59.5% and 34.1% at 12 h and 24 h respectively) as compared to free drug</td>
<td>(Shafie and Fayek, 2013)</td>
</tr>
<tr>
<td>Literature Review</td>
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<tr>
<td><strong>C16Y peptide</strong></td>
<td>Intravital injection</td>
<td>PLA/polyethylene oxide NPs of C16Y peptide were significantly more effective, than free C16Y, in choroidal neovascularization (CNV). (Kim and Csaky, 2010)</td>
<td></td>
</tr>
<tr>
<td><strong>Dexamethasone</strong></td>
<td>Intravital injection</td>
<td>PLGA- NPs sustained the drug release for at least 50 days in the rabbit eyes. (Zhang et al., 2009)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous Injection</td>
<td>Sialyl Lewis X conjugated liposomes showed selective targeting to retina for treatment of autoimmune uveoretinitis. (Hashida et al., 2008)</td>
<td></td>
</tr>
<tr>
<td><strong>DNA</strong></td>
<td>Subretinal injection</td>
<td>PNP formulated with polyethylene glycol (PEG)-substituted lysine 30-mers (CK30PEG) after single or double subretinal injections were found to transfect RPE cells at a higher efficiency than naked DNA. (Han et al., 2012)</td>
<td></td>
</tr>
<tr>
<td><strong>EGFP expression plasmid</strong></td>
<td>Subretinal injection</td>
<td>No signs of local inflammatory response associated with administration of compacted DNA nanoparticles administration was observed. (Ding et al., 2009)</td>
<td></td>
</tr>
<tr>
<td><strong>Flt23K plasmid</strong></td>
<td>Intravenous injection</td>
<td>PLGA-nanoparticles conjugated with transferrin or RGD peptide could significantly reduce CNV lesions as compared to the nonfunctionalized ones. (Singh et al., 2009)</td>
<td></td>
</tr>
<tr>
<td><strong>PEDF peptide</strong></td>
<td>Intravital injection</td>
<td>PLGA- NPs evaluated in a mouse model of retinal ischemia showed a longer protection of the retinal ganglion cell layer with no noticeable side effects upto 7 days. (Li et al., 2006)</td>
<td></td>
</tr>
</tbody>
</table>
| **pEGFP-1 plasmid**                                                                                              | Subretinal injection                                                                                         | PNP showed efficient                                                                                              (Koirala et al., 2009)
<table>
<thead>
<tr>
<th>Plasmid of plasminogen kringle 5 (K5)</th>
<th>Intravitreal injection</th>
<th>PLGA-NPs showed expression for 4 weeks in retinal model of neovascularization post injection.</th>
<th>(Park et al., 2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNA</td>
<td>Subconjunctival injection/ topical</td>
<td>Alexa647-labeled pRNA-NPs (pRNA-X) administered via subconjunctival injection in mice were internalized in retina. Delivery via topical route was however insignificant.</td>
<td>(Feng et al., 2014)</td>
</tr>
<tr>
<td>Rh-6G and Nile red</td>
<td>Intravitreal Injection</td>
<td>PLA- NPs accumulated at the ILM 1h after intravitreal injection, followed by a transretinal distribution 6h after administration. At 18–24h, the PNPs were localized within the RPE cells and were found to be present in the RPE cells for up to four months after injection.</td>
<td>(Bourges et al., 2003)</td>
</tr>
<tr>
<td>siRNA</td>
<td>Intravitreal injection</td>
<td>Pegylated liposome-protamine-hyaluronic acid nanoparticles could</td>
<td>(Liu et al., 2011)</td>
</tr>
</tbody>
</table>
efficiently protect siRNA and the CNV area in the murine model could be reduced more effectively by the nanoparticles as compared to the administration of naked siRNA.

<table>
<thead>
<tr>
<th>Tamoxifen</th>
<th>Intravitreal injection</th>
<th>PEG-coated cyanoacrylate PNP inhibited onset of experimental autoimmune uveoretinitis model. The PNP were retained for 3-9 days post injection.</th>
</tr>
</thead>
</table>

(Kozak et al., 2004)

4.3 Lipidic Nanoparticles

Solid lipid nanoparticles (SLN) comprise a nanosized lipidic core stabilized by a layer of surfactants. They offer several advantages over other colloidal carriers, such as the possibility of controlling drug release, long-term stability, ability to encapsulate hydrophilic or a lipophilic drug, high drug load, absence of biotoxicity due to the use of physiological lipids, possibility of sterilization by autoclaving, and easy large scale production. In addition, due to their nano size and lipidic nature, SLNs can effectively achieve ocular drug delivery by enhancing corneal absorption, improving ocular bioavailability, prolonging the ocular retention time, and providing a sustained drug release profile (Seyfoddin et al., 2010).

The second generation of these lipid systems is the nanostructured lipid carrier (NLC) which overcomes the issue of limited drug loading and drug expulsion usually observed with SLN systems. NLCs consist of a mixture of solid and liquid lipids at room temperature resulting in greater number of imperfections. These imperfections leave sufficient space to accommodate the drug; thereby, the drug-loading capacity and stability is higher and long term, relative to SLN (Tian et al., 2012).

Araujo et al. (2011) developed NLCs containing Nile red. These NLCs were detected in the retina on topical administration reaching a peak at 40 min after administration. Prolonged retention followed by corneal and non-corneal (major) uptake make NLCs a promising approach for achieving selective and prolonged drug concentration in the posterior segment of the eye.
Del Pozo-Rodríguez et al. (2008) reported transfection of DNA-SLN (Plasmid pCMS-EGFP-SLN) in RPE cells with a low transfection efficiency of less than 2.5%. Latter was accredited to clathrin-mediated lysosomal degradation of the vector. Low division rate of retinal cells also hampers the entrance of DNA into the nucleus. However, when DNA was complexed with sweet arrow peptide (SAP) before adsorbing on previously prepared SLNs (del Pozo-Rodríguez et al., 2009), transfection efficiency increased significantly as compared to DNA-SLN (6% and 3.5% respectively). This effect was attributed to shift in the uptake pathway from clathrin endocytosis to caveolae/raft-dependent endocytosis (Fig. 6), thereby reducing vector degradation.

After internalization in the cell and release of DNA, there are two mechanisms to overcome the nuclear envelope: the interruption of the nuclear membrane during mitosis or the entrance through the nuclear pore complex using nuclear localization signals (Duvashani-Eshet et al., 2008). Delgado et al. (2011) complexed DNA with protamine and adsorbed it on SLNs which induced a 6-fold increase (29%) in the transfection capacity of SLNs in retinal cells as compared to DNA-SLN without protamine. The authors reasoned such a high transfection to DNA condensing potential of protamine. The activity reduces exposure to cytoplasmic agents like DNAases and hence stabilizes DNA against degradation. The authors observed that vectors containing protamine require endocytosis via clathrin for transfection. Lysosomal activity induces the release of the complex protamine–DNA from the SLNs, making condensed DNA easily accessible to intranuclear transcription, with protamine helping in its nuclear localization. Likewise, the authors modified DNA-SLN with polysaccharides which significantly increased the transfection of RPE cells, reaching levels close to 50% of transfection (Gasco´N et al., 2012).

Same group (Delgado et al., 2012) used SLNs composed of dextran, protamine, and the plasmid pCEP4-RS1 as non-viral gene vector, which was able to transfect, in vitro, ARPE-19 cells, producing significant amounts of retinosquisin, whose deficiency is responsible for the X linked juvenile retinoschisis. The nuclear localization signals of protamine, its ability to protect the DNA, and a shift in the entry mechanism from caveolae-mediated to clathrin-mediated endocytosis promoted
by the dextran, led to an increase in transfection. On administering these vectors intravitreally, subretinally, and topically, the expression of enhanced green fluorescent protein (EGFP) was monitored. After subretinal injection, the vectors transfected RPE cells as well as photoreceptors while on intravitreal injection, though there was a significant response in the retinal ganglion cells but poor protein expression was observed in the RPE cells. These vectors could also transfect corneal cells after topical application as eye drops.

4.4 Polymeric Nanomicelles

Polymeric micelles are nanosized (10 to 100 nm) self-assembly of amphiphilic block copolymers above critical micellar concentration with a hydrophobic core and hydrophilic shell (Nishiyama and Kataoka, 2006). The shell is responsible for micelle stabilization and in particular circumstances interacts with biomembranes. Amphiphilic block copolymers can be tailored to prolong the stability of micelles in the eye fluids, induce bioadhesion, and modify drug release.

These systems serve as excellent pharmaceutical carriers because of their ability to enhance solubility of hydrophobic drugs, nanosize, presentation as an aqueous dispersion, prevention or minimization of drug degradation, lower adverse side effects and improved drug permeation through ocular epithelia with minimal or no irritation, ultimately leading to enhanced ocular bioavailability (Cholkar et al., 2012).

The most commonly used shell-forming polymers are poly(ethylene glycol) i.e. PEG derivatives which have several advantages such as high aqueous solubility, low toxicity and immunogenicity, capacity to minimize protein adsorption to micelle surface and improve the micelle biocompatibility (Adams et al., 2003). Furthermore, most of the polyoxyethylated nonionic surfactants are Pgp efflux inhibitors that helps in achieving enhanced bioavailability of drugs that undergo extensive Pgp efflux (Pepiæ et al., 2012).

Mitra et al. (2010) formulated nanomicelles consisting of nonionic surfactants with particle size ranging from 10-30 nm. These nanomicelles were found to reach retina following topical application majorly through the conjunctival/scleral pathway by facilitating passive diffusion through the scleral water channels rather than the corneal pathway.
Plasmid DNA with lacZ gene encapsulated in poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) polymeric micelles when administered as eye-drop could achieve efficient transfer of the functional gene to the intraocular tissues such as retinal pigment epithelium, and vitreous body for up to 48h (Liaw et al., 2001). Methoxypoly(ethylene glycol)–poly(β-caprolactone) (MPEG–PCL) is a polymer composed of hydrophilic MPEG segments and hydrophobic PCL segments, that can self-assemble into micelles for hydrophobic drug delivery. Xu et al. (2013) demonstrated safety of MPEG–PCL micelles on RPE cells after intravitreal injection. These polymeric micelles seem to have better stability than surfactant micelles even on intravenous injection (Adams et al., 2003; Yamamoto et al., 2002) due to low critical micellar concentration (1000 fold) values of these polymers (10⁻⁶-10⁻⁷M) as compared to surfactants. Thus they tend to retain their integrity and drug content in eye fluids allowing stability to them for a suitable time to reach the target site before dissociation into unimers.

The surface charge is also an important parameter for the stability and interaction of these micellar structures with biomembranes. However, the interaction of polymeric micelles with components of eye fluids (e.g. proteins, mucin, glycosaminoglycans) and cells also needs to be evaluated in terms of micelle stability and drug release (Pepia et al., 2012). Design strategies for development of these micelles should pay a special attention on the physicochemical properties, composition and dynamics and complex eye environment of the eye fluids. Adsorption of proteins from tear fluids can compromise stability of these systems, leading to their disintegration and decreased transport across biological membranes. Further to this, these systems will also face the anatomical barriers of ocular delivery including immediate wash out from the eye surface which needs to be addressed suitably before formulating them as ocular delivery systems.

Polymeric micelles formed through electrostatic interaction (polyion complex (PIC) micelle) encapsulating fluorescein isothiocyanate-labeled poly-L-lysine after single intravenous administration showed a \( C_{\text{max}} \) at 4 h in retina-choroid and drug was detectable even up to 7 days. The authors attributed such an observation to long-
circulating characteristics of PIC micelles which accumulated in CNV lesions and were retained there for 168 h (Idetaa et al., 2004).

Ideta et al. (2005) encapsulated photosensitizer, dendritic porphyrin (DP) in polymeric micelles for the treatment of exudative AMD with photodynamic therapy. DP loaded PIC micelles were administered by intravenous injection, and after application of a mild laser light, there was selective accumulation of DP in the choroidal neovascularature.

4.5 Nanoemulsions

Nanoemulsions consist of two immiscible liquids in which one liquid is dispersed as droplets in another liquid (Sadurni et al., 2005; Shakeel and Ramadan, 2010) stabilised by the use of surfactants. These homogeneous systems, which can be prepared over a wide range of surfactant concentrations and oil to water ratios, are all fluids of low viscosity, thus applicable for topical administration to the eye. The surfactant in combination with co-surfactant lowers the interfacial tension which ultimately facilitates dispersion process during the preparation of nanoemulsion and provides a flexible film that can readily deform around the droplets (Azeem et al., 2009). Presence of a surfactant and co-surfactant increases membrane permeability, thereby increasing drug uptake. Hence, these systems act as penetration enhancers facilitating their own corneal uptake (Ammar et al., 2009). The choice of surfactant, oil, co-surfactant is important since these ingredients need to be non-irritating and non-toxic to the corneal surface and other ocular tissues.

In addition to this, nanoemulsions provide sustained release of the drug, high penetration in the deeper layers of the ocular structure, ease of sterilization (Ammar et al., 2009), low viscosity, and their capacity to accommodate both hydrophilic and lipophilic drugs. Thus, these systems can achieve a faster therapeutic action with a smaller dose resulting in fewer systemic (due to localized delivery) and ocular side effects. Latter, may also result from a decreased need to repeat the applications per day. This factor also tends to enhance better patient compliance (Vandamme, 2002).

However, only a single study report by Hagigit et al. (2010) proclaimed the protection of oligonucleotide (ODN 17) by its entrapment in nanoemulsion from vitreous degradation and presence in retina even 3 days after its intravitreal injection.
5. Conclusions
Delivering drug in appreciable amount to posterior eye segment necessitates direct but invasive delivery which needs to be repeated regularly over a period of several months to years. Intravitreal route is very aptly described as the most effective treatment option exhibiting worst safety. Not only is it highly invasive as it involves disruption of the globe, but it may manifest in a variety of possible complications which range from retinal detachment and cataract to endophthalmitis in addition to being costly, and painful. Use of nanocarrier systems preferably as ocular drops, with a potential to overcome highly protective anatomical barriers and physiological constraints or as periocular, or intravitreal injections, which can deliver the drug for a prolonged period, of time requiring less frequent administration may be an answer to this problem. The nano-ocular drops combine the benefits of conventional delivery systems i.e., self-administration, patient compliance, convenience and minimized side-effects with enhanced ocular bioavailability, low frequency of administration and prolonged action without being invasive or harmful to the tissue integrity. However, majority of these systems are still in a nascent stage of development and are yet to see the pharmaceutical market due to issues related to the cost of development and manufacture, ability for scale up, and approval by the regulatory authorities. Though a significant research databank has accumulated to fuel the interest and expectations of both the clinicians and the pharmaceutical manufacturing houses yet much is left to be desired, before these systems can eventually be translated to commercially viable options. Inspite of several promising results achieved with ex vivo, preclinical tests and cell line studies, large clinical trials are required to establish the benefits of nanocarrier systems in the real life situations are still awaited except for rare instances of success.

Examples of Visudyne® and Photrex® are encouraging for promoting the use of liposomes for posterior segment delivery, however, both these systems have been administered systemically and require a supplemental phototherapy. The acceptability of these liposomal systems on the other hand is attributable to long experience and expertise of researchers with these systems in addition to the safety of liposomal components. However, it is expected that other nanocarrier systems which are being
explored will also find a suitable place in the ocular drug delivery armamentarium if pursued suitably, provided a safe and proficient evidence of their efficacy is established.