International Journal of Drug Discovery
ISSN: 0975–4423 & E-ISSN: 0975–914X, Vol. 3, Issue 1, 2011, pp-56-62
Available online at http://www.bioinfo.in/contents.php?id=24

THE CHALLENGES OF OPHTHALMIC DRUG DELIVERY: A REVIEW

SINGH V.[1], AHMAD R.[1,2], HEMING T.[2]*
[1]SBS PG Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand, India
[2]Department of Physiology and Biochemistry, Oman Medical College, Sohar, Sultanate of Oman
*Corresponding Author: Email- theming@omc.edu.om

Received: February 06, 2011; Accepted: March 19, 2011

Abstract- During the past decades, researchers involved in the development of ophthalmic pharmaceuticals have understood that ophthalmic drug delivery is a challenging subject for optimization. Normal vision is dependent upon the optimal functioning of ocular barriers and intact membranes that function to selectively regulate the environment of ocular tissues. Novel pharmacotherapeutic modalities have aimed to overcome the biological barriers which can impede efficient ocular drug delivery. To explore the impact of ocular barriers on research related to ophthalmic drug delivery and targeting, herein we provide a review of the literature of work carried out on the biological constraints and new approaches to ophthalmic drug delivery.
Key words: ocular barriers, tear film, ophthalmic drug delivery, cornea, eye

Introduction

"If a physician performed a major operation on a seignior (a nobleman) with a bronze lancet and has saved the seignior's life, or he opened the eye socket of seignior with a bronze lancet and has saved the seignior's eye, he shall receive ten shackles of silver." But if the physician in doing so "has caused the seignior's death, or has destroyed the seignior's eye, they shall cut off his hand." These excerpts are from King Hammurabi's code, dated to about 100 BC [1].

The eye is a unique organ, both anatomically and physiologically, containing widely varied structures with independent physiological functions. For example, the cornea and the crystalline lens are the only tissues in the body besides cartilage that lack blood supply. The complexity of the eye provides unique challenges to ocular drug delivery strategies. Pharmaceutical interventions and drug delivery methods for treating eye diseases and disorders vary considerably depending on the nature and extent of the disease or disorder [2]. Some ocular diseases (e.g., hordeolum, blepharitis) are associated with tissues at the front of the eyes and, hence, are amenable to treatment with topically-applied drugs. Other diseases such as diabetic retinopathy and age-related macular degeneration are associated with tissues at the back of the eye, which are less accessible to topically-applied drugs.

Traditional ocular drug delivery methods include solutions, ointments, and suspensions. These methods account for nearly 90% of available ophthalmic formulations in the United States [3]. Of these formulations, almost two-thirds are solutions. Generally, these products are delivered via an eye-drop bottle, which relies on gravity as the primary motive force to propel the drop into the eye.

Typically, the bioavailability of drugs applied in eye-drops is very poor. Ocular absorption of topically-applied drugs is limited by protective mechanisms that promote proper functioning of the eye, as well as by a number of concomitant factors related to the efficacy of drug application [3]. Firstly, the topically-applied drug is immediately diluted in ocular tear liquid. Secondly, excess solution spills over the lower eyelid, with some of the remaining drug draining into the nasolachrymal duct. Drainage by the nasolachrymal system can occur when the volume of fluid in the eye exceeds the normal lachrymal volume of about 7–10 µl. In contrast, the application of 1-2 drops of a drug medication by eye-dropper represents roughly 50–100 µl. Thirdly, after initial dilution, spilling and drainage of a topically-applied agent, any remaining drug can be diluted further by increased lachrymation and physiological tear turnover, induced by the drug application. As a result, the loss of topically-applied drug from the eye can be almost three orders of magnitude greater than the rate of absorption of the drug by the eye.

The cornea is the main route for transport of topically-applied drugs into the eye. Small lipophilic molecules are normally absorbed through the cornea, while large hydrophilic molecules (e.g., protein/gene-based medicines) are absorbed via the conjunctiva and sclera [2, 4]. However, lachrymal drainage and systemic absorption from the conjunctiva act to remove ophthalmic medications from the eye. This results in the ocular absorption of only a small fraction of the topically-applied drug dose [5, 6]. Corneal contact time has been estimated to be in the order of minutes at best, with drug bioavailability less than 10 %. Any remaining

drug is subject to nonselective adsorption into the anterior chamber. In spite of these limitations, ophthalmic solutions are still given high priority by formulators since they are simple to prepare, filter and sterilize.

The emergence of futuristic medicaments (e.g., gene-based medicines) for treatment of ocular diseases demands the development of effective strategies to enhance drug bioavailability [7, 8]. Both *in vivo* animal models and *in vitro* cell-based models have been employed for such investigations. Animal-based experiments are important for pharmacological and/or toxicological studies while cell-based models are relevant to mechanistic investigations [8, 9]. Various animal models including rabbits, pigs, dogs, cats, mice, rats, and monkeys have been exploited for pharmacokinetic and bioavailability studies. The rabbit model is most commonly used despite morphological and biochemical differences with the human eye such as lower blinking rate, larger corneal and conjunctival surface area, and the absence of melanin pigments in the anterior uvea of albino rabbits. Such differences may significantly influence the results of ocular pharmacotherapeutic research. Furthermore, animal experiments have been extensively criticized in terms of cost, time and ethical issues [9]. *In vitro* cell-based models have been used for studying ocular barrier functions as well as cellular uptake and transport machineries. Such models also can be used for cellular and molecular studies (e.g., cellular metabolism and biomarker detection) related to developing novel therapeutic modalities (e.g., genome-based therapeutics, monoclonal antibodies, nanobodies).

Tear Film

The primary physiologic obstacle against topically-applied drugs is the tear film. The tear film is the first protective layer of the cornea and conjunctiva. It contains optimal electrolyte composition, pH and nutrient levels, and a complex mixture of proteins, lipids, and mucin. The tear film consists of three layers: the outermost lipid layer (0.1 µm in thickness secreted by meibomian glands), a middle aqueous layer (7-10 µm in thickness), and the innermost mucous layer (0.2–1.0 µm in thickness). The contents of tear film are secreted by various glands of the eye and corneal epithelial cells [10]. The tear film influences cellular migration and normal cell differentiation, wound healing, ocular secretion of electrolytes and water, and ocular immunity. These functions involve a wide variety of growth factors, cytokines and biologically-active peptides including epidermal growth factor, hepatocyte growth factor, transforming growth factor, basic fibroblast growth factor, tumor necrosis factor, and granulocyte-macrophage colony stimulating factor. Major tear proteins that display antibacterial/antiviral activities include lysozyme, secretory immunoglobulin, lactoferrin, lipocalin, peroxidase, high-molecular weight glycoproteins, and mucins. Other bioactive substances present in tear film include interleukins, substance P, and endothelin 1 [10].

Corneal and Non-Corneal Routes of Absorption

The mechanical and chemical barrier functions of the cornea control the access of exogenous substances into the eye, thereby protecting intraocular tissues. The human cornea measures approximately 12 mm in diameter and 520 µm in thickness, and consists of five layers; epithelium, basement membrane (Bowman's layer), stroma, Descemet's membrane, and endothelium. The human corneal epithelium is a stratified, squamous, non-keratinized epithelium, 50 µm in thickness. It is composed of 2-3 sheets of flattened superficial cells, wing cells, and a single sheet of columnar basal cells. The tightest monolayer is made by the outer superficial epithelial cells which display tight junction complexes (*Zonulae occludens*). These tight junctions seal the superficial cells, building a diffusion barrier in the surface of the epithelium. In contrast, the basal cells are separated by 10–20 nm intercellular spaces. The wing and basal cells communicate via gap junctions allowing the intercellular communication of small molecules. Compared to the stroma and endothelium, the corneal epithelium represents a rate-limiting barrier which hinders permeation of hydrophilic drugs and macromolecules.

The stroma and Descemet's membrane cover the inner endothelial cells. These cell layers contain macula adherens and are more permeable than the epithelium. The stroma displays an hydrophilic nature due to an abundant content of hydrated collagen, which prevents diffusion of highly lipophilic agents.

The corneal endothelial monolayer maintains an effective barrier between the stroma and aqueous humor [11]. Active ion and fluid transport mechanisms in the endothelium are responsible for maintaining corneal transparency [12].

The cornea is an important absorption route of many topically-applied drugs. Certain drug properties such as lipophilicity, molecular weight, charge, and degree of ionization significantly influence a drug's passive permeability across the cornea [13]. Of these factors, lipophilicity plays a key role since transcellular permeation of lipophilic drugs through the cornea is faster and greater as compared to hydrophilic drugs. Greater molecular size decreases the rate of paracellular permeation of drugs [14, 15]. Once in the cornea, the drug can diffuse into the aqueous humor and the anterior segment. However, local administration of conventional drugs via the corneal route fails to provide adequate concentrations within the vitreous and retina [16, 17].

The major intraocular route for entry of topically-applied macromolecules and hydrophilic substances is the conjunctiva. The conjunctiva is a mucous membrane consisting of vascularized epithelium (2-3 cell layers thick) and plays an important role as a protective barrier on the ocular surface since tight junctions are present on the apical surface of its cells. In fact, the bulbar conjunctiva represents the first barrier against permeation of topically-applied drugs via the non-corneal route. Drugs absorbed via the conjunctiva are rapidly removed from the eye to the systemic circulation, with resultant poor bioavailability to ocular tissues [18].

The sclera is a secondary route for entry of topically-applied macromolecules and hydrophilic substances. The sclera has about half the permeability of the conjunctiva to such molecules. The sclera is poorly vascularized and consists mainly of collagen and mucopolysaccharides, through which drugs can diffuse and enter the posterior segment of the eye (uveal tract, retina, choroid, vitreous humor).

Blood-Ocular Barriers

Systemic/intravitreal application is the main route of drug administration for many posterior segment disorders, by which adequate concentrations of drug can be achieved and maintained in the retina and vitreous. However, oral and intravenous routes can impose unwanted side effects due to the use of high doses to compensate for the fact that only a small fraction of the drug reaches ocular tissues across the blood-ocular barriers. Two blood-ocular barrier systems control the movement of solutes and nutrients into inner ocular tissues; the blood-aqueous barrier (BAB) and blood-retinal barrier (BRB). The balancing of inflow and outflow of aqueous humor across these barriers controls intraocular pressure. Blood-ocular barriers can be overcome using intravitreal injection. However, drawbacks to intravitreal injection include the risks of endophthalmitis, lens damage, retinal detachment, and low compliance.

Blood-Aqueous Barrier: The BAB is located in the anterior part of the eye, and is formed by endothelial cells of the blood vessels within the iris, as well as the nonpigmented cell layer of the ciliary epithelium. Both cell layers contain tight-junction complexes. Nonetheless, the barrier has measurable permeability to macromolecules. It has been shown that macromolecules such as horse radish peroxidase are able to permeate the fenestrated capillaries of the ciliary body, despite the fact that such molecules cannot permeate the iris blood vessels [19]. Functions of the BAB are important for maintaining transparency of the ocular media and the chemical equilibrium of ocular fluids [20].

The BAB allows small lipophilic drugs to enter the uveal blood circulation and, consequently, facilitates their rapid elimination from the anterior chamber. In contrast, larger and more hydrophilic drugs are merely eliminated by aqueous humor turnover across the BAB [20].

Blood-Retinal Barrier: The BRB is located in the posterior part of the eye and is composed of two cell types, namely the retinal capillary endothelial (RCE) cells and retinal pigment epithelial (RPE) cells which form the inner and outer BRB, respectively. The inner BRB covers the lumen of retinal capillaries and protects the retina from circulating molecules in the blood circulation. Unlike the choroidal capillary endothelial cells, RCE cells possess intercellular tight junctions which are formed by intercellular communications of RCE and glial cells [21]. Immunostaining studies for the tight junction protein occludin reveals a high degree of well-organized tight junctions in retinal arterioles and capillaries. This supports a role of astrocytes in formation of tight junctions within RCE cells. *In vitro* studies have shown that astrocyte-conditioned media supplemented with cAMP inducers can dramatically increase the barrier properties of cultured endothelial cells, suggesting that a soluble component may lead to barrier properties [22]. The ability of glial cells to influence endothelial barrier properties suggests that disruption of the BRB in ocular diseases could be related to functional changes in glial cells and/or the retinal vascular endothelium. Because of the functional expression of tight junctions and intercommunication with glial cells (astrocytes and Müller cells), the biological characteristics of RCE cells are similar to the blood-brain barrier (comprised of brain capillary endothelial cells, pericytes, and astrocytes) [23]. Despite these similarities, the density of interendothelial junctions and vesicles are greater in retinal vessels as compared to the brain. Accordingly, passive diffusion of a vascular tracer has been shown to be significantly higher in the retina than the brain of rats [24]. While the inner BRB is permeable to lipophilic substances, this barrier displays poor permeability for proteins and small hydrophilic compounds [12].

The outer BRB displays tight barriers due to the presence of tight junctions (*Zonula occludens*). Specialized transport processes within the RPE together with robust barrier restrictiveness of RPE control the traverse of nutrients/compounds, allowing selective exchange of nutrients between the choroid and retina [5, 17]. Polarized RPE cells display a predominantly apical localization of Na^+,K^+-ATPase which regulates intracellular Na^+ and K^+ homeostasis [25, 26]. *In vitro* studies by Steuer *et al.* [27] with isolated primary porcine RPE tissue demonstrated that the cells are poorly

permeable to macromolecules and small hydrophilic compounds, but not to lipophilic compounds.

Satisfactory delivery and efficient pharmacological effect of drugs within the vitreous and retina require systemic or intravitreal drug administration. Systemic application via oral or intravenous administration, however, requires high doses of the drug since blood flow and barrier properties of the BRB allow very small fractions of the drug to reach the posterior chamber; typically only 1-2% of the plasma concentration. Therefore, a large proportion of the drug is disseminated within the entire body potentially leading to unwanted consequences [28]. Loss of normal BRB function is a common feature to many retinal degenerative disorders (e.g., in diabetic patients). Thus, the development of therapies to prevent the loss of barrier properties or to restore these properties is a high priority in ophthalmology.

Vitreous Body

The vitreous body occupies a volume of about 4.5 ml and is the largest single structure in the eye, contributing 80% of total ocular volume. It supports the retina, and is probably essential for preservation of crystalline lens clarity. The vitreous body is a gel containing more than 99% water, stabilized by collagen fibrils, glycosaminoglycans, and proteoglycans. The intact vitreous acts as a barrier against the bulk movement of solutes. High concentrations of antioxidants, such as ascorbic acid, can therefore accumulate in the vitreous and might protect the lens against oxidative damage [27]. The ability of the vitreous to prevent bulk movement of solutes depends on the degree of liquefaction of the gel. Although it is difficult to assess the degree of vitreous liquefaction by ophthalmic imaging techniques, diffusion within the vitreous of a tracer substance (e.g., fluorescein) can be illustrated *in vivo* by vitreous fluorophotometry in humans.

Direct intravitreal injection entails the obvious advantage of being able to achieve immediate therapeutic concentrations in the eye while largely avoiding systemic exposure. Nevertheless, drugs are rapidly eliminated from the vitreous, typically by first-order kinetics [29]. Thus, repeated injections are needed to retain therapeutic concentrations in the eye, but are associated with risks of endophthalmitis, cataract formation, and retinal detachment. To sustain sufficient intraocular drug levels after intravitreal injection, studies have examined the prolonged delivery of drugs via liposomal systems incorporating small and large unilamellar vesicles with half-lives of approximately 10 and 20 days, respectively [30].

It appears that both anterior and posterior routes are involved in the elimination of drugs from the vitreous. Active transport machineries and/or passive diffusion are responsible for the drug elimination. The anterior route involves drainage into the anterior chamber followed by clearance via bulk aqueous flow, while the posterior route involves active or passive permeation across the retina and RPE followed by systemic dissipation. Following intravitreal drug administration, high drug lipophilicity or the presence of an active transport mechanism leads to rapid transport across the retina into systemic circulation. Therefore, longer vitreous half-life can be observed when drug passage through the BRB is not possible and the drug has to diffuse into the anterior chamber first to be removed either by aqueous flow or by diffusion across the iris. For instance, gentamicin and penicillin are removed from the vitreous via the anterior chamber and by crossing the retina at rates of 0.035 h^{-1} and 0.18 h^{-1}, respectively. This difference clearly highlights the impact of these different elimination routes [31].

Ocular Membrane Transport Machineries

Cell membranes represent a barrier to free movement of molecules between extra- and intracellular compartments. A solute, based on its molecular properties, can be transported across cell membranes by passive/active transport, carrier-mediated transport, or receptor-mediated transport (endocytosis and transcytosis) [32]. Most ocular tissues, such as corneal epithelial and endothelial cells, display Na^+/H^+ exchanger, Na^+/HCO_3^- symporter, and Cl^-/HCO_3^- exchanger which are involved in the regulation of intracellular pH [33]. The Na^+/H^+ exchanger is present in the basolateral membranes of both epithelial and endothelial cells of the cornea, while the Na^+/HCO_3^- transporter is localized to the basolateral aspect of the corneal endothelium and only slightly expressed in the corneal epithelium. This implies that apical and basolateral membrane distribution of ion transporters serves cellular needs and physiologic functions [34].

Influx and Efflux Transporters

Influx and efflux transport machineries are functional in major membranous barriers including the cornea, conjunctiva, iris, ciliary body, and retina [35]. Uni- or bi-directional influx transporters such as monocarboxylate transporters, glucose transporters, amino acid transporters, and peptide transporters supply essential nutrients. Among the ATP-binding cassette superfamily of transporters, the P-glycoprotein (P-gp) and multidrug resistance associated proteins (MRPs) play a key role in unidirectional efflux of substances. Human and rabbit corneal epithelium, significantly express P-gp and MRPs [36]. Similarly, such “efflux pumps” have been identified in different ocular tissues including retinal capillary endothelial cells, RPE cells, non-pigmented ciliary epithelium, conjunctival epithelial cells, and iris and ciliary endothelial cells [37, 38].

Endocytosis and Transcytosis

Specialized receptors exist within ocular barriers which control the passage of xenobiotics. Endocytosis pathways via clathrin-coated or caveolae (non/smooth coated) vesicles account for receptor-mediated transport in ocular tissues. Expression of clathrin and the integral protein of the caveolae domain, caveolin-1, has been reported in ocular tissues [39]. Using cultured human retinal pigment epithelial cells, as well as a mouse model, Mo *et al*. [40] showed involvement of caveolae-mediated endocytosis pathways in the uptake of albumin nanoparticles. However, Qaddoumi *et al*. [41] reported that endocytosis of poly(lactic-co-glycolic)acid nanoparticles in primary cultured rabbit conjunctival epithelial cells occurs mostly independent of clathrin- and caveolin-1-mediated pathways, despite mRNA and protein expression of clathrin.

Advances in Ophthalmic Drug Delivery

Recent advances in topical ocular drug delivery have ranged from improvement of primitive eye-drops to iontophoretic drug delivery, *in situ* gelling systems, dendrimers, penetration enhancers, lipid emulsions, ocular inserts, muco-adhesive and thiolated polymers, stimuli insensitive hydrogels, and site-specific drug delivery systems. All such endeavors aim at enhancing drug bioavailability by providing prolonged or sustained delivery to the eye or by facilitating transcorneal penetration. Nonetheless, very few drug delivery systems have successfully appeared on the market: currently, 95% of products are delivered via the traditional eye-drop bottle.

The first approach made towards research in the field of improving the ocular contact time of solutions utilized the incorporation into an aqueous medium of polymers, such as polyvinyl alcohol, polyvinyl pyrolidine, methylcellulose, carboxymethyl cellulose, or hydroxypropyl cellulose. The increased solution viscosity reduced the solution drainage from the eye. For example, increasing the solution viscosity of pilocarpine solution from 1 to 100 cps by incorporation of methylcellulose reduced the solution drainage rate constant 10-fold and resulted in a 2-fold increase in pilocarpine concentration in the aqueous humor [42]. An optimal viscosity of 12-15 cps has been suggested for ocular drug absorption by Paton and Robinson [43]. Natural polymers including sodium haluronate and chondroition sulfate are being investigated as viscosity-inducing agents. Prolonged residence time with an extended duration of action for 1% pilocarpine has been observed with 0.2-0.3% sodium hyaluronate solutions [44]. The use of a bioadhesive polymer (e.g. chitosan, hyaluronic acid, poly acrylic acid) that prolongs the residence time in the precorneal region may be advantagous [45, 46]. Other promising approaches focus on development of drug delivery systems that dispense medication as a mist below blink- and lachrymation-thresholds (OptiMyst) [47] or as multidoses of very small volumes; 12–15 µl range (Versidoser) [48].

Another new method involves the use of a low electrical current, administered through a removable temporary applicator placed under the lower eyelid, to transport an ionized drug to eye tissues [49]. Advanced technology based on the use of nanocarriers (nanoparticles, liposomes, dendrimers) has been investigated recently with the aim of enhancing ocular drug delivery. These systems are claimed to provide a prolonged residence time at the ocular surface, minimizing the effect of natural eye clearance systems [50, 51]. Advanced methods for subcutaneous delivery can range from injections to sustained release implants, but can be associated with greater risk of infection, internal ocular bleeding and retinal damage.

Conclusion

Ophthalmic drug delivery requires optimization because risk cannot be tolerated in regards to the eyes. A substantial amount of work has been carried out to develop new drug delivery systems which are efficient in delivering accurate and precise doses with minimum toxic effects. Still, more optimization is required in ophthalmic drug delivery. In order to optimize drug delivery systems, it is important to consider effective corneal application to promote good corneal penetration, prolonged contact time with the corneal epithelium, and the use of a drug solution with appropriate rheological properties that is non-irritable to the eye in order to limit lachrymation and reflux blinking.

The challenges for effective ophthalmic drug delivery include non-biologic, as well as biological constraints. Any improved delivery system must circumvent the physics of eye-drop delivery, which can result in chronic overdosing, which in turn can produce unwanted adverse effects. The system should minimize the use of preservatives in the drug solution being applied. The system also should avoid application of excess volumes of drug solution, which then drain through the nasolachrymal duct into the circulatory system with potential systemic absorption. The cells and tissues of the eye are restrictively regulated to maintain optimal visual function. For such unique specialized functions, tight cellular barriers in the anterior and posterior segments of the eye play a key role by selective control of the inward and outward traverse of fluids and solutes. These barriers also effectively control the shuttling of administered drugs. Hence, effective drug delivery and targeting is faced by challenges to overcome these barriers.

List of Abbreviations

BAB blood-aqueous barrier
BRB blood-retinal barrier

P-gp	P-glycoprotein
MRPs	multidrug resistance associated proteins
RCE	retinal capillary endothelial
RPE	retinal pigment epithelial

References

[1] Majno G. (1975) *The Healing Hand - Man and Wound in the Ancient World. Harvard University Press, Cambridge, MA*, 43-45.

[2] Macha S. and Mitra A.K. (2003) *Overview of ocular drug delivery. In: Mitra A.K. (editor) Ophthalmic Drug Delivery Systems, 2nd ed. Marcel Dekker, New York*, 1-12.

[3] Saettone M.F. (2002) *Pharmatech Business Briefing*, 167, 171.

[4] Ahmed I. (2003) *The noncorneal route in ocular drug delivery. In: Mitra A.K. (editor) Ophthalmic Drug Delivery Systems, 2nd ed. Marcel Dekker, New York*, 335-363.

[5] Singh V., Bushetti S.S., Raju A., Shareef A., Imam S.S. and Singh M. (2010) *Indian J Ophthalmol*, 58(6), 477-481.

[6] Mitra A.K., Anand B.S. and Duvvuri S. (2006) *Drug delivery to the eye. In: Fischbarg J. (editor) The Biology of the Eye. Academic Press, New York*, 307-351.

[7] Barar J., Javadzadeh A.R. and Omidi Y. (2008) *Expert Opin Drug Deliv*, 5, 567-581.

[8] Urtti A. (2006) *Adv Drug Deliv Rev*, 58, 1131-1135.

[9] Hornof M., Toropainen E. and Urtti A. (2005) *Eur J Pharm Biopharm*, 60, 207-225.

[10] Dartt D.A., Hodges R.R. and Zoukhri D. Tears and their secretion. In: Fischbarg J. (editor) The Biology of the Eye. Academic Press, New York, 21-82.

[11] Huang H.S., Schoenwald R.D. and Lach J.L. (1983) *J Pharm Sci*, 72, 1272-1279.

[12] Sunkara G. and Kompella U.B. (2003) *Membrane transport processes in the eye. In: Mitra A.K. (editor) Ophthalmic Drug Delivery Systems. Marcel Dekker, New York*, 13-58.

[13] Schoenwald R.D. and Huang H.S. (1983) *J Pharm Sci*, 72, 1266-1272.

[14] Huang A.J., Tseng S.C. and Kenyon K.R. (1989) *Invest Ophthalmol Vis Sci*, 89(30), 684-689.

[15] Hamalainen K.M., Kananen K., Auriola S., Kontturi K. and Urtti A. (1997) *Invest Ophthalmol Vis Sci*, 38, 627-634.

[16] Janoria K.G., Gunda S., Boddu S.H. and Mitra A.K. (2007) *Expert Opin Drug Deliv*, 4, 371-388.

[17] Duvvuri S., Majumdar S. and Mitra A.K. (2003) *Expert Opin Biol Ther*, 3, 45-56.

[18] Ahmed I. and Patton T.F. (1985) *Invest Ophthalmol Vis Sci*, 26, 584- 587.

[19] Freddo T.F. (2001) *Exp Eye Res*, 73, 581-592.

[20] Cunha-Vaz J.G. (1997) *Doc Ophthalmol*, 93, 149-157.

[21] Gardner T.W., Antonetti D.A., Barber A.J., Lieth E. and Tarbell J.A. (1999) *Doc Ophthalmol*, 97, 229-237.

[22] Barar J. and Omidi Y. (2008) *J Biol Sci*, 8, 556-562.

[23] Omidi Y., Barar J., Ahmadian S., Heidari H.R. and Gumbleton M. (2008) *Cell Biochem Funct*, 26, 381-391.

[24] Stewart P.A. and Tuor U.I. (1994) *J Comp Neurol*, 340, 566-576.

[25] Lin H., Kenyon E. and Miller S.S. (1992) *Invest Ophthalmol Vis Sci*, 33, 3528-3538.

[26] Quinn R.H. and Miller S.S. (1992) *Invest Ophthalmol Vis Sci*, 33, 3513-3527.

[27] Steuer H., Jaworski A., Stoll D. and Schlosshauer B. (2004) *Brain Res Protoc*, 13, 26-36.

[28] Selvin B.L. (1983) *South Med J*, 76, 349-358.

[29] Ashton P. (2006) *Retinal drug delivery. In: Jaffe G.J., Ashton P. and Pearson P.A. (editors) Intraocular Drug Delivery. Taylor & Francis, New York*, 1-25.

[30] Guidetti B., Azema J., Malet-Martino M. and Martino R. (2008) *Curr Drug Deliv*, 5, 7-19.

[31] Yasukawa T., Ogura Y., Tabata Y., Kimura H., Wiedemann P. and Honda Y. (2004) *Prog Retin Eye Res*, 23, 253-281.

[32] Omidi Y. and Gumbleton M. (2005) *Biological membranes and barriers. In: Mahato R.I. (editor) Biomaterials for Delivery and Targeting of Proteins and Nucleic Acids. CRC Press, New York*, 232-274.

[33] Jentsch T.J., Matthes H., Keller S.K. and Wiederholt M. (1985) *Pflugers Arch*, 403, 175-185.

[34] Gao J., Sun X., Yatsula V., Wymore R.S. and Mathias R.T. (2000) *J Membr Biol*, 178, 89-101.

[35] Mannermaa E., Vellonen K.S. and Urtti A. (2006) *Adv Drug Deliv Rev*, 58, 1136-1163.

[36] Karla P.K., Pal D., Quinn T. and Mitra A.K. (2007) *Int J Pharm*, 336, 12-21.

[37] Wu J., Zhang J.J., Koppel H. and Jacob T.J. (1996) *J Physiol*, 491(Pt 3), 743-755.

[38] Saha P., Yang J.J. and Lee V.H. (1998) *Invest Ophthalmol Vis Sci*, 39, 1221-1226.

[39] Lo W.K., Zhou C.J. and Reddan J. (2004) *Exp Eye Res*, 79, 487-498.

[40] Mo Y., Barnett M.E., Takemoto D., Davidson H. and Kompella U.B. (2007) *Mol Vis*, 13, 746- 757.
[41] Qaddoumi M.G., Gukasyan H.J., Davda J., Labhasetwar V., Kim K.J. and Lee V.H. (2003) *Mol Vis*, 9, 559-568.
[42] Paton T.F. and Robinson J.R. (1976) *J Pharm Sci*, 65, 1295-1301.
[43] Camber O., Edman P. and Gury R. (1987) *Curr Eye Res*, 6, 779.
[44] Zeimer R.C., Khoobehi B., Niesman M.R. and Magin R.L. (1988) *Invest Ophthalmol Vis Sci*, 29, 1179.
[45] Nagaresenker T.J., Londhe V.Y. and Nadkarni G.D. (1999) *Int J Pharm*, 190, 63-71.
[46] Singh V., Raju S.A., Bushettii S.S., Javed A. and Singh M. (2010) *Ind J Pharm Edu Res*, 44(3), 380-385.
[47] www.optimystsystems.com
[48] www.mysticpharmaceuticals.com/Drug Delivery Technology/Ophthalmic Delivery.htm
[49] El Jarrat-Binstock E. and Domb A.J. (2006) *J Controlled Release*, 110(3), 479-489.
[50] de Campos A.M., Diebold Y., Carvalho E.S., Sanchez A. and Alonso M.J. (2004) *Pharm Res*, 21(5), 803-810.
[51] Spataro G., Melecaze F., Turrin C.O., Soler V., Duhayon C., Elena P.P., Majoral J.P. and Carminade A.M. (2010) *Eur J Med Chem*, 45(1), 326 -340.