Permeation through cornea

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The permeability of the cornea to drugs is clinically important because it is the major factor determining the efficacy of topically applied ophthalmic preparations. With this perspective, the present article gives a brief update and overview of corneal structure and proposed mechanisms of permeation. Physiological, physicochemical and formulation factors affecting drug permeation through cornea are highlighted. Influence of ocular penetration enhancers on drug permeation is also discussed.

The use of medical therapeutics in the treatment of eye diseases is as old as medicine itself. Ocular therapy has progressed inexorably but by a circuitous path. The earliest account of ophthalmic treatment on record dates back to the Mesopotamian era, when Imhotep (2,800 BC), an Egyptian physician used malachite topically, in the treatment of trachoma. In seventh century BC, powders were blown into the eye through reeds and tubes as a means of topical administration. The Greek physician Pedacus Dioscorides (40-90 AD) compiled five-volume materia medica De Universa Medicina that gave first therapeutic delivery system, Collyrium, for ophthalmic medication. However, improvement in ocular delivery preparations and topical treatments did not occur for many centuries with ocular therapeutics only finding meaningful value in the seventeenth century until when the understanding of transcorneal permeation was sketched.

The permeability of the cornea to drugs is clinically important because it is the major factor determining the efficacy of topically applied preparations. Therefore, an integrated knowledge of the relevant anatomical and physiological constraints that impede or modify ocular drug and vehicle disposition through cornea is important.

Corneal structure

The cornea is a transparent, avascular tissue, approximately 0.5-0.7 mm thick and about 11.5 mm in diameter. Broadly, cornea is divided into 3 layers: epithelium, stroma and endothelium. Epithelium is a tight junction tissue that is about six cell layers thick. Physiologically, the epithelium is relatively impervious to polar or hydrophilic compounds with relative molecular weight greater than 60-100 Da. Glucose (Mol. Wt. =180 Da), for example, does not pass through the epithelium. Lipophilic compounds pass the epithelium due to solubilization in the lipid cell membranes, while it provides major resistance to the movement of ions or water. The anterior corneal stroma is condensed into a thin membrane known as Bowman's membrane. The stroma constitutes 85-90% of the cornea and consists of fine collagen fibrils and mucopolysaccharides able to hold a substantial quantity of water. Fibrils are spaced so that the cornea is transparent when at normal thickness. Loosely attached to the posterior surface of the stroma is another interfacial layer known as Descemet's membrane. The stroma is readily transversed by water soluble, polar compounds and less so by non-polar compounds. Even high molecular weight substances diffuse with ease. The innermost layer of the cornea, the endothelium, consists of a single cell layer which houses an active water pump that regulates corneal thickness through hydration. It is a very porous tissue with an open intercellular network and thus large molecules (up to 70,000 Da) can traverse this membrane easily.

Mechanisms of permeation through cornea

There are two major pathways for the movement of compounds through the corneal tissue: transcellular and paracellular. Transcellular drug movement involves cell/tissue partitioning/diffusion, channel diffusion and carrier mediated transport. In contrast, paracellular represents diffusive and convective

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transport occurring through intercellular spaces and tight junctions. Diffusive transport is a dissipative process that depends upon the difference in the solute concentration and the permeability-surface area properties of the membrane while, convective transport is dominated by a balance of hydrostatic and osmotic gradients, solute concentration and hydraulic and reflection coefficient of the restrictive barrier\(^\text{16}\). Partitioning of a compound across cellular membranes is especially important for hydrophobic molecules and is represented by a relatively high activation energy for diffusion. While aqueous diffusional channels are important for corneal transport of hydrophilic compounds and are characterized by low activation energy\(^\text{17}\).

The cellular arrangement of epithelium of cornea precludes paracellular transport of most ophthalmic drugs and limits lateral movement within the anterior epithelium\(^\text{19}\). Corneal surface epithelial intracellular poresize has been estimated to be about 60 Å (ref. 19). Small ionic and hydrophilic molecules appear to gain access to the anterior chamber through these pores\(^\text{20}\), however, for most drugs, paracellular transport is precluded by the tight interjunctional complexes. Stroma exerts a diffusional barrier to highly lipophilic drugs owing to its hydrophilic nature. There are no tight junction complexes in the stroma and paracellular transport through this tissue is possible. The endothelial permeability depends on molecular weight and not on the charge or hydrophilic nature of the compound\(^\text{21,22}\).

The general mechanisms\(^\text{23-26}\) of drug movement through cornea are given below:

A. Organ level
- Rate-limiting membrane for most drugs is the corneal epithelium which acts both as a barrier to penetration and as a reservoir for drug.
- The rate-limiting barrier for most drugs appear to reside in the top two cell layers of the epithelium.
- Stroma is rate limiting for very lipid-soluble drugs.

B. Cellular level
- Small molecules, for example, water, methanol, ethanol, propanol, and butanol, readily traverse the cornea through assumed aqueous pores. Their permeability constants are very large.
- Water-soluble compounds traverse the cornea by the paracellular route. The smaller the partition coefficient, the smaller is the permeability constant.
- Peptides, ions and other charged compounds appear to penetrate the cornea by paracellular route.
- Substances that possess biphasic solubility traverse the cornea more easily.
- Lipid soluble substances pass readily through the limiting cellular membranes. They do not penetrate in proportion to their concentration.

Factors affecting drug permeation through cornea

Majority of ocular preparations are formulated in aqueous vehicle. The factors that largely determine ocular bioavailability of drug from aqueous formulation, may be divided into 3 categories:

A. Physiological factors
B. Physicochemical factors
C. Formulation factors

A. Physiological factors

The loss of drug from the precorneal area is a net effect of drainage, tear secretion, non-corneal absorption and corneal absorption rate processes. Collectively these processes lead to typical corneal contact time of about 2-4 min in humans, for an instilled solution and an ocular bioavailability that is commonly less than 10% (ref. 27).

1. Precorneal factors—Various precorneal factors causing loss of drug are:

   (a) Tear turnover—Tears wash out at a rate of 16% per min, except during periods of sleep or during anaesthesia. Normal tear volume is only 7 µl, so drug loss is substantial.

   (b) Instilled solution drainage—The precorneal area can hold approximately 30 µl, including resident tears when the eye is not blinking. The volume reduces to 10 µl in blinking eye. Therefore, excess of instilled volume either spills or rapidly drains from the eye into the nasolacrimal duct with subsequent absorption into the systemic circulation. Drainage of an instilled drug solution away from the eye is responsible for a considerable loss of drug and hence affects the biological activity of drugs in the eye. The rate of this drainage is related to the volume of drug solution instilled and increases with increasing volume. The drainage rate of an instilled volume increases at a rate proportional to the volume of the fluid in the eye more than the normal lacrimal volume. The drainage rate is about 100 times faster than the corneal absorption rate\(^\text{28}\). Lee and Robinson\(^\text{29}\) gave following equation to predict drainage:
\[ V_t = V_o + V_i e^{-K_d t} \]

where, \( V_t \) = volume remaining in the conjunctival sac at time \( t \), \( V_o \) = normal lacrimal tear volume, \( V_i \) = volume of instilled drop, and \( K_d \) = drainage rate constant. Drainage rate constant is directly proportional to the volume of instilled drop i.e. smaller the drop instilled, the lower the drainage rate and greater the extent of ophthalmic absorption.

Instilled volume – In the rabbits, 90% of the dose is cleared within 2 min for an instilled volume of 50 \( \mu \)l, 4 min for an instilled volume of 25 \( \mu \)l, 6 min for an instilled volume of 10 \( \mu \)l and 7.5 min for an instilled volume of 5 \( \mu \)l. This volume dependency of solution drainage rate has been found to exert its expected effect on the percent of dose absorbed into the eye and on the pharmacological effect that follows. The effect of reducing the instilled volume but keeping the dose constant was evaluated from the miotic effects of pilocarpine nitrate in rabbits by Chrai et al.29. As the volume of the instilled drop was reduced from 50 to 25, 10 or 5 \( \mu \)l, the area under the miosis-time curve increased 1.2, 1.8 and 3-fold, respectively. In the same study, it was shown that instillation of 10 \( \mu \)l of a 2% epinephrine solution to rabbits caused the same pupillary response as 50 \( \mu \)l of a 10% solution. The same principle was demonstrated by Patton and Francœur30, who found equal bioavailability for pilocarpine nitrate after instillation of 25 \( \mu \)l of 0.01 \( M \) solution (67.8 \( \mu \)g) or 5 \( \mu \)l of 0.092 \( M \) solution (26 \( \mu \)g). This resulted in a 2.6-fold improvement in bioavailability from a 5-fold reduction in dropsize. Brown and coworkers31,32 extended the benefits of this phenomenon to the clinical use of phenylephrine, a highly effective mydriatic, but with significant cardiovascular risk in certain patients. Eleven neonates were administered 8 \( \mu \)l of 2.5% phenylephrine hydrochloride in one eye and 30 \( \mu \)l in the other eye. The mean pupillary diameters (4.86 and 4.57 mm respectively) were the same. However, when the said volumes were administered to two different groups of infants, the plasma concentration of phenylephrine was 0.9 ng/ml for 8 \( \mu \)l dose and 1.9 ng/ml for the 30 \( \mu \)l dose31. Clinical application of the benefits of small-volume dosing has also been reported33,35 for dosing mydriatic solutions to children and adults. In their studies, these authors found equal dilations of the pupil in patients receiving 0.005 ml ointment or drop dosage form of phenylephrine, cyclopentolate or tropicamide hydrochloride salts and in patients receiving 50-75 \( \mu \)l of repeated aqueous drops of these medications. These studies suggest that there is an advantage to small-volume dosing owing to an improvement in ocular bioavailability. This allows a reduction in dose and a reduction in the potential for systemic side effects. Keister et al.36 reported that reducing the instilled volume would increase only the ocular bioavailability of drugs with low permeability and would not effect the ocular bioavailability of drugs with high corneal permeability. Therefore, the clinical implication is that appropriate reduction of instilled volume and the simultaneous increase in instilled drug concentration permit substantial dosage reductions without reduction in absolute dose of the drug37. Chrai et al.38 studied the effects of dilution and drainage on the absorption of pilocarpine nitrate and epinephrine hydrochloride in rabbits when dosed topically in different concentrations and volume and at different dosing intervals. The results indicated that two drops instilled immediately after one another showed a reduced bioavailability compared to separating the doses by 3-5 min. This implies that multiple dosing is more efficient when sufficient time is allowed to elapse between instillations. The volume dependency of the drainage rate has also been observed with suspensions39 but not with liposomes. Lee et al.40 reported that liposomes were cleared from the conjunctival sac of albino rabbits with approximately the same first order rate constant (0.45 min\(^{-1}\)) over the instilled volume range of 10-50 \( \mu \)l. The size and number of liposomes are more important factors than instilled volume influencing the extent of ocular drug absorption from liposomes.

(c) Protein binding—Tears normally, contain about 0.7% protein\(^{41}\) and the protein level increases during infection or inflammation. Unlike the blood, where the drug-protein complex continues to circulate, tears are replaced quickly thus removing both free and bound forms of the drug. Mikkelson et al.\(^{41}\) showed that the miotic response to topically applied pilocarpine was reduced about 2 times as the albumin concentration in the precorneal fluid was increased from 0-3%.

(d) Non-productive drug absorption—Upon instillation, drug is absorbed into the cornea and conjunctiva. The surface area of the conjunctiva is about 17 times that of the cornea\(^{42}\) with 2-30 times greater permeability to many drugs\(^{43}\). All tissue absorption other than the cornea is perceived as non-productive loss when the target tissue is the interior of the eye.
This loss can be minimized in two ways: Varying drug lipophilicity or changing the drug formulation. Formulation changes that are most effective in minimizing the ratio of conjunctival to corneal drug absorption are increasing solution pH, lowering solution tonicity and lowering the concentration of EDTA and benzalkonium chloride in the formulation. The changes in lipophilicity and drug formulations have a greater effect on corneal than conjunctival penetration. Chast et al. reported the pharmacokinetics of a single dose of morphine ocularly applied in rabbits before and after lacrimal canalculi ligation and results suggested a great capacity of conjunctiva for drug reabsorption.

It is important to mention here, the assumption that drug absorbed by the conjunctiva is swept into systemic circulation is incorrect. Series of experiments conducted by Doane et al. and Ahmed et al. proved the same. They postulated that the drug absorbed by the conjunctiva could lead to direct entry into the uveal tract, bypassing the cornea and this route of drug entry into the eye is called non-corneal route.

2. Membrane factors—Membrane factors include area available for absorption, thickness, porosity and tortuosity of the cornea and lipophilicity/hydrophilicity balance. The cornea consists of three layers, with respect to barrier resistance, namely the epithelium, stroma and endothelium. Permeability studies on excised corneas have indicated the superficial layers of epithelium as the primary rate-determining barrier for penetration of both water soluble and lipid soluble drugs. Because the epithelium is lipophilic, low in porosity and relatively high in tortuosity and thickness, a rapidly penetrating drug must possess log partition coefficient greater than 1 in order to achieve a sufficient penetration rate. Although both epithelium and endothelium are considered lipophilic, measurements of the water permeability of each layer indicate that endothelium is 2.7 times more permeable than the epithelium. Studies on excised corneas by Kim et al. and Maurice indicate that non-electrolyte penetration through the endothelium occurs primarily through the intercellular spaces. The stroma is basically acellular, hydrophilic in nature, high in porosity and low in tortuosity but because it represents 90% of the thickness of the cornea, the stroma is significant in overall contribution to resistance. Huang et al. determined the resistance to permeability of each corneal layer for a group of β-blocking agents. The results indicated epithelium as the rate determining barrier for hydrophilic compounds and stroma for lipophilic compounds. When absolute values were compared, the lipophilic compounds were found to have greater permeability coefficients. Authors suggest that penetration through stroma occurs when drug diffuses through an aqueous media of gel-like mucopolysaccharide interspersed by a matrix of collagen fibrils.

Kedem and Katchalsky analysed the permeability characteristics of biological membranes using thermodynamics and concluded that three independent coefficients, e.g. permeability of the solute, hydraulic conductivity and reflection coefficient, were necessary to describe the permeability properties of a membrane. Hydraulic conductivity involved a net volume flow of water driven by either hydrostatic or osmotic pressure gradients. The reflection coefficient was dependent on the interactions of the solute and water with the membrane and gave a measure of the relative semipermeability of a membrane. Mishima and Hedhys estimated the hydraulic conductivity of the epithelium and endothelium of the rabbit cornea and the reflection coefficients of these layers with various solutes. The hydraulic conductivity of the epithelium and endothelium was found to be approximately 1.0x10^{-5} and 2.3x10^{-5} mm/min msOml respectively. The reflection coefficient of the epithelium was found to be 1 and that of endothelium between 0.6-1.

Rojanasakul and Robinson investigated corneal permselectivity by measuring membrane electokinetic potentials generated either by ionic concentration gradient (diffusion potential) or hydrostatic pressure gradient (streaming potential). Studies indicated dual-selective character to passage of ions across the cornea. The magnitude and polarity of the selectivity are controlled by the degree of protonation of ionizable sites within the cornea. The cornea possesses an isoelectric point of 3.2. At physiologic pH and pH above the isoelectric point, the cornea behaves as a negatively charged membrane and allows preferential passage of positive ions in comparison to negative ions. Below isoelectric point, the reverse is true. A study of the in vitro flux of lysine (MW 146, positively charged) and glutamic acid (MW 147, negatively charged) indicated an approximately two to three-fold difference in the permeability of the two compounds with lysine being more absorptive. Lowering the ionic strength of the bathing solution
caused an increase in membrane effective charge density due to lesser degree of electrostatic shielding which resulted in a significant increase in both membrane resistance and selectivity.60

Due to its dual capability to terminate the pharmacological activities of inherently active drugs and to transform inactive drugs to their active moieties, drug metabolism in the eye is an important aspect of drug action. Drugs that are degraded by oxidation or reduction are less likely to be metabolized in the eye than those that are degraded by hydrolysis.62 Cornal epithelium and the iris-ciliary body are metabolically active due to presence of esterases, aminopeptidases, and ketone reductase. Drugs that contain ester linkage are likely to be hydrolysed by the esterases. After the topical application of pilocarpine, to the rabbit eye, when the miotic effect is at its peak, up to 50% of the drug is hydrolysed to pilocarpic acid by the esterases present in the intercellular spaces of cornea and aqueous humor. Esterases play a very important role in the activation of ester prodrugs.64 Susceptibility of these prodrugs to esterase-mediated hydrolysis is an important factor that affects not only the onset of drug action but also the extent of corneal penetration. The lipid soluble drug, dipivefrin, is transported faster across the cornea than the parent molecule, epinephrine. Subsequent to the transport, the inactive prodrug is presumably hydrolysed to active epinephrine in the anterior chamber by esterases.65

B. Physicochemical factors

Physicochemical factors are the major determinants to passive diffusion across the cornea.

1. Partition coefficient — Partition coefficient (between octanol/water) is a parameter for quick assessing of penetration potentials of drugs into different biological membranes. Corwin Hansch described linear or parabolic relationship between drug effectiveness and the partition coefficient (lipophilic character) of the drug. A parabolic graph identifies an optimal partition coefficient at its apex. Partition coefficient-penetrability correlation is helpful in the design of optimally permeable ophthalmic drugs. Schoenwald and Ward determined rates of permeability across excised rabbit corneas for 11 steroids and a plot of permeability vs. log partition coefficient resulted in a parabolic relationship with optimal permeability at log partition coefficient of 2.9. Narukar and Mitra observed parabolic relationship between partition coefficient and corneal permeability of 5' aliphatic esters of 5 iodo-2'-deoxyuridine. In vitro corneal permeability was optimum at log partition coefficient of 0.88. Likewise, Mosher and Mikelson determined the in vitro corneal transport of n-alkyl-p-aminobenzoate ester homologues and observed a parabolic relationship with an optimal permeability at a log partition coefficient of 2.5-2.6. Similar parabolic relationship between corneal permeability and partition coefficient has been reported for β-blocker whereas Wang et al. described a sigmoidal relationship for the same. However the guiding criteria for design of prodrugs for enhanced corneal permeation have been the parabolic relationship.

In any study, a measure of the corneal penetration efficiency of drugs is the partition coefficient. The percentage contribution of each corneal barrier layer against transport of β-blocking agents and a few other drugs across excised rabbit cornea has been studied. The results show that for hydrophilic drugs (log partition coefficient < 0), the epithelium provides a large percentage of the resistance to corneal penetration. For lipophilic drugs with log partition coefficient between 1.6-2.5, the stroma contributes a significant percentage of the resistance. And for drugs with log partition coefficients between 0-1.6, the sum of the stromal and endothelial resistances equals the epithelial resistance. Kishida observed in vitro relationship between transcorneal permeability of drugs and their physicochemical properties and found that the transcorneal permeabilities of hydrophobic and biphasic soluble substances were dependent on their lip solubility.

Thus an optimal lipophilic/hydrophilic balance in the molecular structure of the penetrant must be achieved to affect rapid penetration through the lipophilic and hydrophilic barriers of the cornea.

2. Solubility — The maximum penetration rate attainable by a drug permeating the cornea is a multiplicative factor of permeability coefficient and tear solubility. If a drug is poorly soluble, its concentration in the precorneal tear film may be limited and therefore its rate of absorption may not be high enough to achieve adequate concentration for therapeutic activity and reverse is also true.

3. Ionization constant — The pKa of ionizable drugs is an important factor in corneal penetration. The degree of ionization influences the extent of diff-
fusion across a membrane. Many drugs are weak acids or weak bases and therefore are partially ionized at physiological pH. The average pH of the tears is 7.2 and the pKa of the drug if within 1 or 2 units of this value, the corneal penetration would be more because major proportion of the administered dose would be in the unionized form. The ionized form of the drug is minimally lipid soluble and if this fraction is too large, the rate of corneal penetration may not be sufficient to produce therapeutic levels of the drug in the eye. Henderson-Hasselbalch equations relate the degree of ionization of a weak acid or weak base to pH of the medium and pKa of the drug as follows:

$$pKa - pH = \log \frac{f_u}{f_i} \quad \text{(for an acidic drug)}$$

$$pKa - pH = \log \frac{f_i}{f_u} \quad \text{(for a basic drug)}$$

From these equations one can determine the amount of unionized drug available for transcorneal movement. The greater the fraction of drug present in the unionized form, the greater is the extent of passive diffusion. Chyi-Fu et al. proved the assumption that only unionized drugs cross the membrane incorrect. Their studies showed that ketorolac (an anion) could permeate through excised rabbit cornea both in unionized and ionized forms. Permeation studies using excised goat cornea also showed similar results for ketorolac, ibuprofen and flurbiprofen.

4. Molecular weight – Molecular weight is related to the diffusional forces active during corneal permeation. For small molecules, the diffusion coefficients are inversely related to the square roots of their molecular weight, while for large molecules, the diffusion coefficients in water are inversely related to the cube roots of their molecular weights. The changes in molecular weight show an inverse relationship to permeability. Compounds with a molecular weight greater than 500 Da offer poor corneal penetration because passive diffusion no longer remains the predominant mode of drug transfer. Compounds of molecular weights less than 500 Da generally diffuse through both biological and synthetic membranes. Molecular weight is a less critical factor since most ophthalmic drugs have a relatively small and narrow range of molecular weight. Exceptions to this may be the higher molecular weight antibiotics such as bacitracin (MW 1411), colistimethate sodium (MW 1250), colistin sulphate (MW 1250), and polymyxin (MW 1200) which penetrate the cornea of disease models but not those of normal rabbit eyes. The transcorneal permeabilities of hydrophobic substances and biphasic-soluble substances are not governed significantly by their molecular weight but by their lipid solubility, while that of hydrophilic substances is governed by their molecular weight. Liaw et al. measured the transport characteristics of series of different molecular weight polyethylene glycols (PEG) and found that corneal absorption of PEG’s showed a dependence on molecular weight with cut-off between PEG 400 and PEG 600.

C. Formulation factors

1. Concentration – Corneal penetration enhancement can be achieved by increasing the solution concentration of a drug, resulting in improved therapy. Davis et al. studied the effect of increasing concentration of tobramycin on Pseudomonas keratitis. The results showed that a 10-fold reduction in number of corneal colonies could be achieved by increasing the concentration of topically applied tobramycin from 4 to 40%. The driving force behind this enhancement is the concentration gradient described by Fick’s first law of diffusion. However increasing the applied concentration to overcome poor clinical efficacy works only till the drug has reached a plateau of its dose-effect curve. Drance and Nash studied the effect of instillations of increasing concentration (1, 2, 4 and 8%) of pilocarpine hydrochloride on intraocular pressure. Maximum reduction in pressure was obtained with 4% solution. The 8% solution though showed the increase in duration, but not an increase in pressure reduction.

Also, increasing concentration may result in hypertonic solutions, which are potentially uncomfortable and can induce increased lacrimation which can accelerate the drainage rate and reduce percent absorption. Ramer and Gassett showed that the extent of ocular absorption for 10% (hypertonic) pilocarpine hydrochloride was only 3-fold greater than that of the 2% solution. In a similar study, Asseff et al. showed that in comparison with 1% pilocarpine hydrochloride, a 2-fold increase in corneal penetration occurred with 4% drops, whereas only a 2.5-fold increase was produced with an 8% (hypertonic) preparation.

2. Particle shape, size and dissolution rate—Suspensions are widely used in ocular drug therapy to administer sparingly soluble drugs or complexes of soluble drugs or to obtain a slow dissolution and a
prolonged release of the drug. Ophthalmic suspensions are generally gently deposited in the cul-de-sac. The drug particles deposited on the eye surface are moved by the eyelids at each blink, which could cause an abrasion of the outer epithelium layers. These may also cause irritation of sensory nerves in the epithelium. The irritation could elicit reflex blinks and reflex lacrimation. Concentration, shape and particle-size together interact to determine the irritation potential of the suspended particles. Forms with sharp angles and edges are more irritant than isometric particles with obtuse angles and edges. Commercial suspensions are formulated to contain nearly spherical particles less than 10 μm in size. Increase in drug particle size influences bioavailability inversely. Three suspensions of 0.1% tritiated dexamethasone were evaluated with varying mean particle size of 5.75, 11.5 and 22.0 μm. As the particle size increased, the in vivo dissolution rate decreased to the point that the particles were removed from the conjunctival sac before dissolution was complete. Both the rate and extent of dexamethasone penetration into the anterior chamber of the rabbit eye decreased.

In another study, with fluoromethalone (a sparingly soluble compound), it was found that the use of a higher concentration of equivalent particle-size did not improve the aqueous humor drug concentration-time profile and absorption of the drug was regulated by its inherent dissolution property. The dissolution rate of the drug is an important parameter which determines the amount of drug actually in solution and thus available for transport through the cornea during the short residence time in the eye. Although the range of particle-size that could be used for ocular suspensions is limited, it is well known that a decrease in particle-size leads to an increased surface area and thus an increase in dissolution rate. Though the surface specific dissolution rate of a sparingly soluble drug increases with a decreasing particle-size, the particle-size has no significant influence on the ocular permeation and that permeability rate rather than the dissolution rate represents the rate-limiting step.

3. pH and Tonicity—The pH for human tears ranges between 7.14-7.28. Tears possess a relatively weak buffer capacity of approximately 3.6×10⁻⁵ (refs 92, 93). The osmolality of the lacrimal fluid is mainly dependent on the number of dissolved ions and crystals. During sleep, the osmolality of the tears varies between 280-293 mOsm/kg and when eyes are open during day, it varies between 302-318 mOsm/kg. On instillation of a hypotonic solution, the permeability of the epithelium is increased considerably and reverse is true for hypertonic solution. The instillation of a hypotonic drug solution creates an osmotic gradient between the tear film and the surrounding tissues. The corneal epithelium is extremely tolerant to large variations in pH and tonicity. The sodium permeability of the epithelium is unchanged from pH 4-10 (ref. 63). Outside this range, epithelial permeability increases especially when bathed with more alkaline solutions. Depending on the drop size, solutions with an osmolality lower than 100 or 266 mOsm/kg and higher than 480 or 640 mOsm/kg are irritant. Ramselkar et al. found that human corneal fluorescein permeability was unaffected in a pH range from 4.5 to 7 and tonicity ranging from isotonic (270 mOsm) to hypertonic (620 mOsm). Conrad et al. reported that alkaline pH induced greater lacrimation than acidic pH in the albino rabbit. This is consistent with the lower buffer capacity of tears in the basic than in the acidic range. Since tears are poorly buffered, induced lacrimation can be minimized by reducing the tonicity of the solution. Hind and Goyan discussed in qualitative terms, significant aspects of the use of buffers in ophthalmic solutions and reported buffer concentration or buffer capacity (index) as an important factor in formulation of ophthalmic solutions containing ionizable drugs. The concentration of the buffer in an instilled solution upon mixing with precorneal fluid dictates the time course of pH, drug ionization and therefore drug absorption. By progressively reducing the buffer concentration of a pH 4.5 citrate buffer from 0.11 to 0 M, Mitra and Mikkelson observed a 5-fold increase in the ocular bioavailability of pilocarpine. Similar results were reported by Ahmed and Patton for ocular penetration and precorneal disposition of pilocarpine from solutions containing different phosphate buffer concentrations at pH 4.5. Seig and Robinson studied the effect of vehicle pH on the absorption of pilocarpine and reported lower absorption with decrease in pH due to pH-induced lacrimation which increased as the pH of the instilled solution was adjusted to values away from physiological pH. However, Conrad et al. suggested that the mechanism responsible for the decreased ocular availability of pilocarpine from instilled solutions of lower pH was...
due to both pH-partition effect and pH induced lacrimation, the magnitude of which depends not necessarily on the pH of an instilled solution, but on the concentration of the buffer contained in an instilled solution. In addition to buffer concentration, the buffer type used also affects the absorption efficiency of topically applied drugs. Phosphate buffer has a high residual buffer capacity and yields a lower bioavailability of pilocarpine than does an acetate buffer. Bioavailability from solutions containing no buffer was maximum and those containing citrate buffer was minimum while acetate and phosphate gave intermediate response. Pretreatment of the eye with a sterile drop of isotonic buffer (pH 9.2), to temporarily alter the tear pH reduces the necessary dose approximately 10-fold for tropicamide, homatropine hydrobromide, phenylephrine hydrochloride and cyclopentolate hydrochloride.

The corneal endothelium is far more sensitive to changes in both pH and tonicity. The optimum endothelial pH is 7.4 to 7.5. Below pH 6.7 and above pH 8.5, the electrical potential difference across the endothelium reaches zero and both structural and functional alterations occur to the endothelium. The endothelium can withstand variations in the tonicity from 200-400 mOsm.

4. Viscosity — It is generally believed that inclusion of a viscoizing agent in an ophthalmic solution will increase ocular bioavailability of the drug due to prolonged residence time of an instilled dose in the conjunctival sac. The more commonly used viscoizing agents include water soluble polymers like polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and cellulose polymers. Methylcellulose was first used in ophthalmology to increase the viscosity of aqueous ophthalmic solutions. In 1965, PVA was introduced to ophthalmology and it was proved that PVA does not blur vision. In 1968, studies on hydroxypropyl methylcellulose (HPMC), a derivative of methylcellulose, showed it to be a superior viscoizing agent than PVA. Chrai and Robinson reported that the rate of solution drainage decreased with increasing viscosity and over a range of 1-15 cps viscosity, 3-fold change in the drainage rate constant was obtained. A further 3-fold change over the viscosity range of 15-100 cps was also observed. The drug bioavailability however, was not proportional to contact time. Even with as much as 100-fold increase in viscosity and a 10-fold decrease in drainage rate, the maximum improvement in drug activity, be it miosis, inhibition of infection or aqueous humor levels is about twice that of an aqueous solution. The decline in preconeral drug concentration is of the first order and rate of decline is proportional to the viscosity of the instilled solution. A linear relationship between the first order drainage rate constant and both the miotic activity and aqueous humor drug levels was obtained over a range of 1-15 cps solution viscosity in studies conducted by Chrai and Robinson. The optimum viscosity to use is in the range of 12-15 cps beyond which the gain in ocular absorption would be minimal, while the risk of inaccuracy of instillation and blurring of vision would increase. Even with a 100-fold increase in viscosity, the gain in ocular drug absorption is modest, being less for oil-soluble than for water-soluble drugs.

A large number of drug studies in humans and animals have been conducted with the use of said polymer vehicles and the results have been expressed in terms of mydriasis or miosis, inhibition of infection, increased contact time, aqueous humor levels of drug, intraocular pressure, and Studies comparing the various polymer vehicles have attempted to emphasize the superiority of one vehicle over the other.

The instillation of water-soluble polymers either change the physiological processes, such as drainage or alters the physicochemical parameters that govern the tear film stability. Some viscosity enhancers, exhibit surface activity, hence interact with the lacrimal film and alter the spreading characteristics of the preconeral tear film, the break-up time, the blinking rate and consequently the elimination of the drug instilled. In general, these polymer vehicles impart a slight lowering of surface tension and an increase in viscosity to the tear film. A lowering of surface tension improves drug mixing with the tear film and its subsequent penetration. Benedetto et al. demonstrated that retention of drug in the preconeral tear film was not strictly because of viscosity of the vehicle or its surface wetting properties but because of surface spreading characteristics and water dragging capacity of the viscoizing agent. Ludwig and Van Ooteghem reported that vehicles of similar viscosity but different surface tension showed no significant differences of AUC values and that physiochemical characteristics as well as concentration of the polymer used affected ocular retention.

When a viscous polymer solution is instilled into the cul-de-sac, several processes reduce its viscosity.
Firstly, the viscous solution is diluted by resident tears and subsequently by incoming tears. Secondly, the viscous solution undergoes shear thinning during blinking, thereby increasing contact area and facilitating mixing. A comparison of the reduction in solution drainage rate by methyl cellulose and polyvinyl alcohol and the resulting increase in aqueous humor pilocarpine concentration in the albino rabbit\textsuperscript{116,121} suggests that it is the flow properties of the vehicle in question and its viscosity, not the concentration, that determines the effect of polymers on solution drainage and ocular drug absorption. In other words, the rheological characteristics of a polymer are important for the retention pattern on the ocular surface. Saettone et al.\textsuperscript{140} studied the influence of different polymers on the mydriatic response of tropicamide. They found that time profiles of the mydriatic responses indicated a preference for pseudoplastic systems rather than Newtonian systems. Saettone et al.\textsuperscript{140,144} also showed that all polymers yielding same viscosity did not effect ocular drug absorption to same extent. These investigators demonstrated that equiequivalent solutions of CMC, HPMC, PVA and PVP enhance the ocular absorption of pilocarpine as well as tropicamide to different extents in humans. The different activity of these polymers was attributed to their influence on spreading characteristics and the thickness of the medication layer on the precorneal area. Non-Newtonian fluid vehicles undergo rheological changes (thinning) when exposed to shear stress during blinking\textsuperscript{142} while Newtonian vehicles do not. Dilatant fluid vehicles thicken with shear while plastic fluid vehicles thin as with pseudoplastic once the yield value is exceeded\textsuperscript{121}.

Saettone et al.\textsuperscript{143} showed that soluble mucoadhesive polyanionic polymers, like hyaluronic acid, polygalacturonic acid, mesoglycan, carboxymethylchitin and polyacrylic acid, enhanced the ocular absorption of pilocarpine more than PVA of equivalent viscosity. Similar favourable effects with hyaluronic acid over HPMC and polyacrylic acid (carbopol 934 P) over PVA have been reported\textsuperscript{144,145}. Deshpande and Shirokar\textsuperscript{146} reported hydrogels prepared with carbopol 940 to be better than those prepared with sodium CMC and HPMC. Rozier et al.\textsuperscript{147} observed an increased contact time and ocular bioavailability of timolol from gelrite (ion activated, \textit{in situ} gelling polymer) formulation as compared to formulation containing an equiequivalent solution of hydroxyethyl cellulose.

On the positive side, increasing viscosity prolongs contact time and therefore promotes absorption. On the negative side, it can slow diffusion of drug in solution and can create mixing problems of the drug solution with tears. In addition, the polymer may adsorb onto absorptive surfaces, creating a barrier for drug penetration. Further, increasing discomfort due to increasing viscosity may induce lacrimation. Hence optimum selection of viscoizing agent and viscosity of solution is necessary for optimum corneal penetration.

**Penetration enhancers**

The bioavailability of topically applied ophthalmic drugs is usually very low (<10\%) (Ref. 27). Attempts have been made to improve ocular bioavailability of drugs through the use of penetration enhancers such as actin cytoskeleton inhibitors, surfactants, bile salts, chelators, preservatives and ion-pairing salts.

\textit{Actin cytoskeleton inhibitors} – Actin cytoskeleton inhibitors like Cytochalsasin B, act by disruption of actin microfilaments at tight junctions of corneal epithelium resulting in increased permeability at these junctions\textsuperscript{148,149}. Cytochalsasin B increases \textit{in vitro} transcorneal permeation of PEG 400 and PEG 600 and appears to cause minimum cell membrane damage\textsuperscript{39}.

**Surfactants** – Benzalkonium chloride, a cationic surfactant, is often added to aqueous ophthalmic preparations as an anti-bacterial preservative in concentration varying from 0.004 to 0.02%. Benzalkonium chloride has been found to increase the corneal penetration of a number of drugs. For example, increased corneal permeability due to benzalkonium chloride has been reported for pilocarpine\textsuperscript{150}, prednisolone\textsuperscript{150}, chloramphenicol\textsuperscript{151}, dexamethasone\textsuperscript{150}, ketorolac tromethamine\textsuperscript{155}, ibuprofen\textsuperscript{157}, flurbiprofen\textsuperscript{157}, timolol\textsuperscript{151}, cyclopentolate\textsuperscript{151}, atenolol\textsuperscript{152}, betaxalol\textsuperscript{152} and fluorescein\textsuperscript{157}. Electron microscopic findings\textsuperscript{153} showed that cationic surfactants produce breakdown in the cohesion of the epithelium by increasing the width of the intercellular space. They also produce some disruption of the cell cytoplasm presumably by their actions on the plasma cell membranes. The cationic surfactants produce an increase in the width of the intercellular space of the superficial epithelial layers and also cause disruption of the superficial cells indicating that they effect not only the cell membranes but also the intercellular route and hence resulting in increased permeability. Topically
applied benzalkonium chloride (0.005 M), also, produces a loosening in adhesion between the corneal epithelium and stromal tissues. Fu and Lidgate reported that increased corneal permeability of ketorolac, an anionic drug, in presence of benzalkonium chloride was not only because of disruption of the epithelial membrane but also because of formation of more lipid soluble ion-pair between ketorolac and benzalkonium chloride. It has been proposed that competitive inhibition of drug-protein binding by ionic surfactants, also may be partially responsible for the increased corneal penetration. In vitro equilibrium dialysis studies conducted with pilocarpine nitrate, sulfisoxazole and methylprednisolone showed that binding of these drugs to protein components of human tears is inhibited by ionic surfactants. Smolen et al. hypothesized that benzalkonium chloride enhanced the epithelial bioavailability of carbocyl by inducing the release of bound drug from binding sites on the corneal surface.

Sodium lauryl sulphate, an anionic surfactant, also enhances corneal permeability. Studies on most surfactants show a permeability enhancement of the cornea with non-i onic, cationic and anionic surfactants. Marsh and Maurice determined the effect of non-ionic surfactants on the corneal penetration of fluorescein in human subjects. Spans and Tweens were mixed to achieve hydrophilic-lipophilic balance (HLB) values ranging from 2-18. The results showed that single instillation of non-ionic surfactant with a HLB range of 16-17 increased fluorescein permeation. Tween20 with a HLB value of 16.7 showed the greatest effect. While most surfactants followed a pattern of increasing permeability when this HLB value was achieved, some surfactants did not follow this general rule suggesting that chemical specificity also play a role. Taniguchi et al. found that Tween80 increased the rate of dexamethasone penetration across the isolated rabbit cornea. Saetone et al. studied the effect of different penetration enhancers e.g. polyoxyethylene glycol lauryl ether (Brij35), polyoxyethylene glycol stearic ether (Brij78), polyoxyethylene glycol oleic ether (Brij98), heteroglycosides (saponin and digitonin) and benzalkonium chloride on in vitro transcorneal permeation of timolol, levobunolol, and cyclopentolate. Among all the Brij, Brij78 showed the maximum permeation of timolol with minimum corneal damage. Brij78 also increased permeation rates of other two drugs but to a lesser extent. Digitonin, saponin and benzalkonium chloride increased permeation rates of timolol but caused substantial corneal damage. Studies conducted earlier indicate that digitonin, significantly increases corneal permeability but may also cause severe corneal damage. Recently Saetone et al. have reported 0.05% w/w concentration of polyoxyethylene alkyl ethers (Brij 35, Brij 78) as safe and effective permeation enhancers for β-blockers like atenolol and timolol. The study confirms the corneal damaging effect of saponin (0.015% w/w), digitonin (0.005% w/w) and benzalkonium chloride (0.02% w/w).

Surfactants are known to impair corneal wound healing and increased opacity of isolated bovine cornea is related to anionic and non-ionic surfactants but not to cationic surfactants. Bile salts – Bile salts have been reported to enhance the permeability of poorly absorbed drugs through the small intestine, rectum and other mucosal membranes.

Morimoto et al. studied the promoting effects of the trihydroxy bile salt, sodium taurocholate (TC-Na) and the dihydroxy bile salt, sodium taurodeoxycholate (TDC-Na) on in vitro corneal permeability of hydrophilic compounds and macromolecular compounds. The results showed that TC-Na and TDC-Na increased corneal permeability of these compounds. The magnitude of the enhancement by TDC-Na was greater than that by TC-Na. The differences in the physicochemical properties of bile salts like solubilizing activity, lipophilicity and calcium ion sequestration capacity, relates to their permeability enhancing effects. The critical micellar concentration (CMC) of dihydroxy bile salts is generally lower and their aggregation numbers larger than those of trihydroxy salts. Thus the solubilizing activity of TDC-Na is higher than that of TC-Na. The lipophilicity and calcium ion sequestration activity of TDC-Na are also higher than that of TC-Na. These higher physicochemical activities of TDC-Na cause loosening of the tight junctions of corneal epithelial barriers resulting in increased permeability. A recent study indicates 0.05% (w/w) concentration of bile salts (sodium taurodeoxycholate and sodium ursodeoxycholate) as safe and effective ocular permeation enhancers for β-blockers like atenolol and timolol.

Chelators—Disodium edetate (EDTA) is a chelating agent that binds divalent cations such as calcium and magnesium. Ashton showed that EDTA increased rabbit corneal epithelial permeability to sor-
bital. Likewise, rabbit corneal permeability in vivo to polar compounds was also increased in presence of 0.5% EDTA. In epithelia, calcium maintains the intercellular matrix and is therefore, an essential factor in determining the size of potential paracellular routes for drug transport. EDTA binds to calcium present in the tight junctions of epithelia. This results in a decrease in calcium concentration in these junctions and thus decreasing the transepithelial resistance to water soluble compounds. EDTA may also cause severe corneal damage.

Preservatives—Commonly used preservatives in ophthalmic solutions and suspensions are benzalkonium chloride, organomercurials and chlorbutanol. Permeation enhancement effect of benzalkonium chloride has already been discussed. The major disadvantage of the three organomercurials (phenyl mercuric acetate, phenyl mercuric nitrate or thiomersal) are their tendency to deposit mercury in corneal tissues and hypersensitivity that is incurred with these agents. Organomercurials react with the membrane sulfhydryl groups and alter membrane permeability and transport systems. Even low concentration of 0.001-0.005% (w/v) commonly used in ophthalmic preparation causes functional changes. Thiomersal has only a small effect on corneal permeability and does not greatly influence drug penetration at normal concentrations.

Chlorbutanol reduces oxygen utilization in the cornea that results in loosened epithelial adhesions. Chlorbutanol when used at concentrations normally found in ophthalmic solution increases corneal epithelial permeability to drugs.

Ion pairing salts—Transport of ionized molecules across natural membranes can be greatly facilitated by provision of a suitable counter ion. This enhancement is the result of an association of oppositely charged species giving rise to an ion pair. Since ion pairs possess no net charge they are far more lipid soluble than the constituent ions and hence better able to permeate through membranes. Neubert has summarized the enhancement in transport rates of some common drugs across natural and artificial membranes in presence of ion pairing salts. Ion pair formation between anti-inflammatory agent chromoglycate and a quaternary ammonium compound has been found to increase the corneal transport of the drug. Ion pairing with m-chlorobenzyltrimethylphosphonium chloride increases in vivo corneal uptake of chloramphenicol succinate. Similarly, increased permeation (in vitro) of ketorolac by ion pairing with quaternary ammonium compound (dodecyltrimethylammonium bromide) has been observed. Enhanced permeability of benzolamide (sulfonamide) in presence of ion pairing salts e.g. tetraphenylphosphonium chloride, tetraphenylarsonium chloride, and trimethylphenylammonium chloride through excised rabbit cornea has been reported. Ion pairing salts thus offer means of enhancing transcorneal permeability of ionized drugs provided either the ion pairing salt or the resultant ion pair does not damage the cornea.

Thus most of the penetration enhancers while promoting corneal permeation of drugs may also damage the cornea. Recent studies however, indicate non-ionic surfactants like Brij, surface-active bile salts (e.g. TDC-Na) and cytoskeletal modulator (cytochalasin B) as promising agents. However, further studies in vivo are needed to know the practical applicability of these penetration enhancers.

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References
19 Lee V H L, J Controlled Rel, 11 (1990) 79.