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Ocular Delivery of Hydrophilic Compounds

Release From Polymer Matrices and Permeability in Ocular Membranes

Doctoral dissertation

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ABSTRACT

After topical ocular administration, drugs are absorbed into the eye via the anterior membranes of the eye. These membranes include the cornea, conjunctiva and sclera. Traditionally the cornea is considered to be the main route for drug absorption, especially for lipophilic, small molecules. The non-corneal route, via conjunctiva and sclera is important in the case of larger hydrophilic molecules with poor corneal permeabilities. Large molecules pose a challenge if one wishes to deliver peptides, proteins and gene-based drugs to the ocular tissues. Polymeric matrices are prolonged action dosage forms that keep the drug in extended contact with the conjunctival surface and, therefore, may be useful for the administration of biotechnology based drugs. The aim of this study was to obtain mechanistic information on molecular determinants of the ocular absorption barriers and drug release from polymeric matrices.

The cornea and conjunctiva are epithelial membranes with tight junctions that limit paracellular absorption. In this study, the permeation of hydrophilic polyethylene glycols (PEG) through NZW-rabbit cornea, conjunctiva and sclera was investigated. For the analysis of the permeated PEG, a thermospray ionization liquid chromatography-mass spectrometry (TSP-LC-MS) method was developed. In addition, a new effusion-based theory was developed to calculate the pore sizes, pore density and porosity in epithelial membranes such as those present in the cornea and conjunctiva. In effusion, permeability is determined by the probability of the drug molecule hitting the pore, not by drug diffusion in the pore. The pore size calculations were based on PEG permeation. PEG permeated through the conjunctiva about 10-15 times better than through the cornea, because the conjunctiva has larger pores and greater porosity than the cornea.

The enzymatic and physical barrier to a peptide, [D-Ala^2]-methionine enkephalinamide (DAMEA), in the cornea, conjunctiva and sclera was investigated with and without peptidase inhibitors (SCH 39370 and bestatin). The model peptide DAMEA was metabolised extensively without inhibitors. The sclera was over 400 times and conjunctiva over 100 times more permeable to DAMEA than the cornea. However, the permeability of DAMEA increased substantially in the cornea and conjunctiva when the enzyme inhibitors were present, highlighting the importance of the enzymatic barrier in these membranes.

We evaluated Gelfoam® and monoisopropyl ester of poly (vinyl methyl ether/maleic anhydride) (PVM/MA) as polymer materials for constructing ocular inserts. The release of model compounds with different physicochemical properties (pKa, molecular weight, hydrophilicity, and charge) was studied. The matrices were prepared by solvent casting and the release was studied using a rotating disk method. The release of compounds from matrices of monoisopropyl ester of PVM/MA was by erosion of the polymer matrix. The molecular weight of the drug did not affect the release rate. In Gelfoam matrices, the release of compounds was diffusion controlled and, therefore, molecular weight and charge of the compounds did influence drug release chracteristics. These two materials, which have different release mechanisms, might be suitable for delivery of certain drugs, for example peptides, permitting their controlled absorption from the conjunctival sac through the conjunctiva and sclera.

National Library of Medicine Classification: WB 340, WW 166, QV 785
Medical Subject Headings: drug administration routes; drug delivery systems; eye; cornea; conjunctiva; sclera; polyethylene glycols; enkephalins; permeability; porosity; polymers; rabbits.

To all my loving people

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Kuopio, December 1999

Kaisa Mari Hämäläinen

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ABBREVIATIONS

Brij-78	polyoxyethylene-20-stearyl ether
Caco-2	a human colorectal carcinoma cell line
DAMEA	D-[Alanine^2]–Methionine-enkephalinamide
DNP	dinitrophenyl
ED	electrochemical detection
EDTA	ethylenediaminetetraacetic acid
FD	fluorescein isothiocyanate dextran
FITC	fluorescein isothiocyanate
GBR	glutathione bicarbonated Ringer's solution
HPLC	high performance liquid chromatography
μ	ionic strength
MDCK	Madin-Darby Canine Kidney
MS	mass spectrometry
mw	molecular weight
m/z	mass/charge ratio
Papp	permeability coefficient
PEG	polyethylene glycol
pI	isoelectric point
PC	partition coefficient
pKa	dissociation constant
PNP	p-nitrophenyl β-cellopentaoside
PVA	polyvinyl alcohol
PVM/MA	poly(vinyl methyl ether/maleic anhydride)
RGD	Arginine-glycine-aspartic acid
rpm	revolutions per minute
RSD	relative standard deviation
SCH 39370	(N-[N-[1-(S)-carboxyl-3-phenylpropyl]-(S)-phenyl-alanyl]-(S)-isoserine)
SIR	selected ion recording
TER	transepithelial resistance
TRH	thyrotropin releasing hormone
TSP	thermospray
Tyr	tyrosine
Tyr-D-Ala-Gly	tyrosine-D-alanine-glycol
UV	ultraviolet
VEGF	vascular endothelial growth factor
VG	vapour gas
ZO	zonula occludens

LIST OF THE ORIGINAL PUBLICATIONS

This study is based on the following publications referred to in the text by Roman numerals **I-V**.

I Auriola S, Rönkkö K and Urtti A: Determination of polyethylene glycols by high performance liquid chromatography-thermospray mass spectrometry. *J Pharm Biomed Anal* 11: 1027-32, 1993.

II Hämäläinen KM, Kontturi K, Auriola S, Murtomäki L and Urtti A: Estimation of pore size and pore density of biomembranes from permeability measurements of polyethylene glycols using effusion-like approach. *J Control Rel* 49:97-104, 1997.

III Hämäläinen KM, Kananen K, Auriola S, Kontturi K and Urtti A: Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. *Invest Ophthalmol Vis Sci* 38:627-34, 1997.

IV Hämäläinen KM, Ranta V-P, Auriola S and Urtti A: Enzymatic and permeation barrier of D-[Ala^2]-Met-enkephalinamide in the anterior membranes of the albino rabbit eye. *Eur J Pharm Sci* 9:265-70, 2000.

V Hämäläinen KM, Määttä E, Piirainen H, Sarkola M, Väisänen A, Ranta V-P and Urtti A: Roles of acid/base nature and molecular weight in drug release from matrices of gelfoam and monoisopropyl ester of poly(vinyl methyl ether-maleic anhydride). *J Control Rel* 56:273-83, 1998.

CONTENTS

1 INTRODUCTION

Usually, drugs are applied topically to the eye as aqueous or oily drops, ointments, gels, or solid inserts (Urtti and Salminen, 1993). Systemic drug treatment of ocular diseases, e.g., as oral administration of tablets is not preferred, because only a small fraction of the dose enters the eye due to the blood-eye barriers and the large volume of drug distribution in the body. Nevertheless, the eye has some physiological and anatomical properties that limit drug absorption also after topical ocular application. These factors include the drainage of the eyedrop solution from the ocular surface, rapid drug absorption into the systemic circulation via conjunctiva, and poor permeability in the ocular barrier membranes (cornea, sclera, conjunctiva) (Fig. 1). In most indications, the drugs must gain access to the interior parts of the eye to exert their therapeutic activity.

For most drugs the cornea is the main pathway of drug absorption into the inner eye (Doane et al., 1978). Usually, the epithelium of the cornea presents the main barrier to drug absorption (Fig 1). It is well known that the conjunctiva of the eye plays an important role in the systemic absorption of topically applied drugs (Fig. 1)(Urtti et al., 1985). However, many studies suggest that the conjunctival/scleral pathway (so called non-corneal route) is an important route of the absorption of hydrophilic and large molecules into the eye (Ahmed and Patton, 1985; Ahmed et al., 1987; Chien et al., 1990).

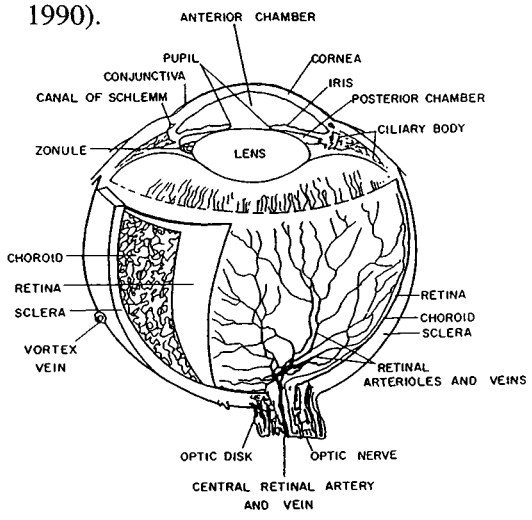

Figure 1. The structure of the eye.

Ophthalmic inserts can be used to prolong ocular contact and, thereby, increase drug absorption. These dosage forms are usually in contact with the conjunctiva and, therefore, they may improve the ocular drug absorption especially through the conjunctiva and sclera (Urtti et al., 1988). Polymer matrices are one example of these controlled release systems. They allow a long duration of action and, in the case of an erodible or soluble polymer, the patient does not have to remove them from the eyes.

Peptides, proteins and gene-based drugs offer an abundance of therapeutic possibilities to treat ocular diseases, but physical and chemical properties (hydrophilicity, charge, and large molecular size) decrease their absorption. This may limit their use in medical therapy. Drugs, which have therapeutic potential in ocular diseases, include cyclosporine and α-interferons that are potentially useful in providing immunosuppression (Banga and Chien, 1988). Various growth factors have been investigated as potential therapeutic agents in wound healing and in neuronal rescue e.g. arginine-glycine-aspartic acid (RGD) containing peptides and anti-sense oligonucleotides (against vascular endothelial growth factor (VEGF)) inhibit neovascularization, and atriopeptin can decrease intraocular pressure (Banga and Chien, 1988; Stratford et al., 1988; Friedlanderet al., 1996; Hammes et al., 1996, Saha et al, 1998). The number of potential targets for peptides, proteins and gene-based drugs will no doubt increase substantially with the completion of Human Genome Project in 2003.

The purpose of this study was to gain understanding on drug release and permeation that should help later in the ocular delivery of peptides, proteins and gene-based drugs. In this study, the permeation routes and metabolic barriers in the rabbit cornea and conjunctiva were characterised. Furthermore, determinants of drug release from polymeric inserts were investigated.

2 REVIEW OF THE LITERATURE

2.1 Topical ocular drug absorption

The most common method of drug treatment in ocular diseases is to instill an aqueous eyedrop into the lower conjunctival fornix. The site of drug action may be on the surface tissues (e.g. in conjunctivitis), but most ophthalmic drugs must permeate through the ocular tissue barriers (cornea, conjunctiva, and sclera) to exert their therapeutic activity (e.g. in glaucoma, intraocular inflammation and infection). After instillation of an eyedrop, less than 5 % of the applied dose is absorbed into the intraocular tissues (Lee and Robinson, 1979). This is due to the tightness of the corneal epithelium, rapid drainage, tear turnover, blinking and non-productive absorption to the conjunctiva as illustrated in Fig 2.

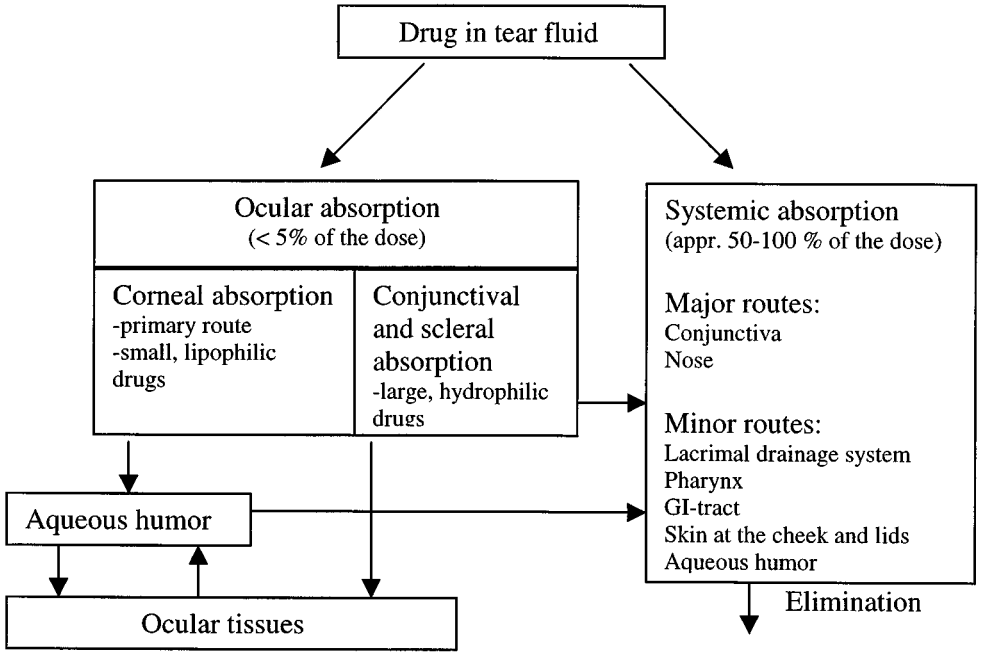

Figure 2. Schematic presentation of ocular absorption related to topical ocular drug administration.

2.1.1 Corneal permeation

Generally, the cornea is the main route of drug absorption from the tear fluid into the eye (Doane et al., 1978; Lee, 1990). The cornea consists mainly of the epithelium, stroma, and endothelium (Harris et al., 1992). The total thickness of the cornea is 500-600 μm, of which approx. 90 % is stroma. The epithelium is 5-6 cell layers in thickness, and it is considered to be the main permeation barrier for hydrophilic drugs (Huang et al., 1983; Chien et al., 1988). The corneal epithelium is lipophilic cellular tissue and the zonula occludens (ZO, tight junctions) completely surrounds the superficial cells of epithelium sealing the paracellular space (Claude, 1978). In epithelia, tight junctions limit permeation of molecules between the cells (Claude, 1978; Gumbiner, 1987). Lipophilicity is a prerequisite for the transcellular permeation in the corneal epithelium (Schoenwald and Huang., 1983). Permeation of hydrophilic drugs is limited to the paracellular space and, in some cases, they may utilise the active transport (Kompella et al., 1995; Hosoya et al., 1998).

The corneal permeation of drugs has been studied extensively. Physicochemical drug properties affect the route and the rate of permeation in the cornea. Corneal permeability is dependent on the octanol-water partition coefficient (PC) (Prausnitz and Noonan, 1998). Usually, the permeability increases with increasing lipophilicity and PC, but it must be remembered that the optimum logPC for corneal drug absorption is between 1 to 2, any further increase in PC will decrease permeability (Schoenwald and Ward, 1978). Therefore, parabolic (Chien et al., 1990) and sigmoidal (Wang et al., 1991; Saha et al., 1996) relationships between the lipophilicity and corneal permeability have been reported.

The molecular size and shape of the drug are important factors that affect the permeation of drugs in the cornea. For hydrophilic polyethylene glycols (PEGs), Liaw and Robinson (1992) determined a cut-off for the corneal permeation at molecular weights of 400-600. Sasaki et al. (1997) reported that the permeabilities of hydrophilic molecules thyrotropin-releasing hormone (TRH) mw 362, p-nitrophenyl β-cellopentaoside (PNP, mw 950), FITC-dextrans (FD-4, mw 4400 and FD-10, mw 9400) showed a significant correlation with the molecular weight. In general, permeation in the cornea was poor.

The degree of ionization affects the corneal permeation (Sieg and Robinson, 1977; Rojanasakul et al., 1992; Liaw et al., 1992; Brechue and Maren, 1993). The unionized drugs usually permeate the epithelium more easily than the ionized form of the drug. For example, free pilocarpine base permeated through cornea 2-3 times better than the ionized form (Mitra and Mikkelson, 1988). At a pH above its isoelectric point (pI) of 3.2, the cornea carries a net negative charge, and therefore, its paracellular space is more permeable to positively charged molecules (Rojanasakul and Robinson, 1989). At a pH below pI, the charge selectivity is reversed. At physiological pH, cationic molecules permeate more easily than anions. Accordingly, the cornea was 2-3 fold more permeable to the cationic L-lysine than to anionic L-glutamic acid (Liaw et al., 1992). Rojanasakul and Robinson (1990) showed by using scanning confocal microscopy that the permeation of negatively charged peptide, insulin (~ mw 5700), was excluded from the paracellular route, whereas the permeation of positively charged peptide poly (L-lysine) (mw 15 000- 30 000) was localized in the intercellular spaces. However, the permeation of poly (L-lysine) in the intercellular spaces was found to be limited to the lower layers of the epithelium, confirming the occluding role of the surface intercellular junctions. Intracamerally administered horseradish peroxidase (mw 40 000), has been observed to diffuse freely in the intercellular space between the cells in the various layers of the corneal epithelium the exception being in the region of the superficial layer (Tonjum, 1974).

In addition to the permeation barrier, the corneal epithelium is metabolically active, which may limit permeation of some drugs. This enzymatic barrier is mainly composed of peptidases, proteases and esterases (Lee et al., 1986a; Harris et al., 1992). For example, pilocarpine, epinephrine and levobunolol are partly metabolised during or after ocular absorption. In the case of peptides, this enzymatic degradation may even totally prevent their therapeutic activity (e.g., methionine and leucine enkephalin) (Lee et al., 1986a).

Stroma is a highly hydrophilic tissue containing about 76-80 % water (Huang et al., 1983). The remainder is composed of collagen fibrils and mucopolysaccharide. Due to its relatively open structure, drugs with a molecular weight up to 500 000 can diffuse in the stroma (Maurice and Mishima, 1984). The stromal permeability (P_{app} range 8.0 x 10^{-}

^{9}- 5.7 x 10^{-5} cm/s) showed no apparent dependence on PC (logPC varied –0.72-3.40) and a strong dependence on molecular radius (0.4-5.0 nm) (Prausnitz and Noonan, 1998). If a molecule is sufficiently lipophilic to easily cross epithelium, the stroma appears to become important. Thus, stroma may be sometimes the rate-limiting layer for lipophilic compounds.

The corneal endothelium is considered lipophilic due to its cellular composition (Huang et al., 1983). It consists of one layer of hexagonal cells with tight junctions (Grass and Robinson, 1988a). Tight junctions of endothelium are wider than those in epithelium. Permeability of endothelial layer (P_{app} range 8.3 x 10^{-9}- 1.7 x 10^{-4} cm/s) has been reported to display a strong dependence on both PC (logPC varied –4.70-2.48) and molecular size (radius varied 0.1-6.6 nm) indicating that both transcellular and paracellular routes are important depending on drug (Prausnitz and Noonan, 1998). Corneal endothelial barrier is leaky since both lanthanium ion and horseradish peroxidase are able to permeate through the endothelium (Kaye et al., 1973).

In addition to maintaining corneal transparency via a pump-leak mechanism, the endothelium plays an active role in the transport of certain proteins (by absorptive endocytosis) to supply nutrients to the stroma and to remove metabolites (Raphael and McLaughlin 1990). Absorptive markers transport through the cells.

For hydrophilic molecules, the rate-limiting barrier of the cornea is in the outermost layer of the epithelium. This has been shown in two ways. Large hydrophilic compounds, like FITC-dextrans (FD), do not permeate to the paracellular space of the corneal epithelium from the tear side (Rojanasakul et al., 1990), but they do permeate from the aqueous humor all the way through the cornea except to the outermost epithelial cell layer. This shows that the tight junctions in the surface layer of the corneal epithelium provide the molecular size-limiting barrier of the cornea. Also, Lee et al. (1991) showed by selective surfactant treatments that the permeation rate of hydrophilic β-blockers is controlled by the epithelial surface. In contrast, permeability of very lipophilic β-blockers was controlled by their distribution from the epithelium to the stroma.

The physiological barriers to topical corneal absorption are formidable. Typically, less than 1% of the instilled dose reaches the aqueous humor (Lee and Robinson, 1986).

After absorption into aqueous humor a drug may distribute to the surrounding tissues; iris, ciliary body, and lens. A small fraction of the drug may also permeate further to the posterior chamber and vitreous humor (Maurice and Mishima, 1984). Drugs permeate to the iris and ciliary body easily because these tissues have a porous, leaky surface. The lens is less permeable than iris or ciliary body and, consequently, drug concentrations are typically lower in the lens than in the anterior uvea (Urtti et al., 1990).

2.1.2 Non-corneal permeation

The intraocular permeation through the non-corneal route involves drug permeation through the bulbar conjunctiva and sclera into the iris-ciliary body (Fig 1). This route is important for hydrophilic and/or large molecules, which have poor corneal permeability. The conjunctival and scleral permeabilities are poorly characterised compared to the corneal permeability (Hughes and Mitra, 1993).

Conjunctiva lines the posterior surface of the eyelid (palpebral conjunctiva) and covers the anterior surface of the globe (bulbar conjunctiva) (Chang and Lee, 1987). Its surface area is about 9 and 17 times greater than the surface area of the cornea in rabbits and humans, respectively (Watsky et al., 1988). Conjunctival epithelium can be divided into bulbar epithelium, which is continuous with the limbal zone of the corneal epithelium; fornix epithelium, which is located mainly in the "folding region"; and palpebral epithelium, which is continuous with the epidermis of the eyelid (Wei et al., 1993). All these three conjunctival epithelia are thought to play an important role in forming the physical protective barrier of the conjunctival surface and, by their goblet cell secretions, in contributing to the formation and maintenance of a tear fluid of the ocular surface. Palpebral epithelium is most stratified. The electron microscopic studies of Buck (1986), revealed that the conjunctival epithelium has a much wider intercellular space than the corneal epithelium. The conjunctiva has lower metabolic activity, it is a less lipophilic environment, it is thinner and it has smaller density of microvilli than the cornea (Nichols et al., 1983; Pfister, 1975).

The scleral barrier resembles that of the corneal stroma: it is tough and thick cellular fibrous tissue. Sclera has three layers: episclera, stroma and lamina fusca and it is composed of collagen fibrils with 2.5-230 nm diameters (Kamai and Ushiki, 1991). In

scleral diffusion, two mechanisms play a role, diffusion across the aqueous media of the gel-like mucopolysaccharides and partitioning into its collagen fibrils (Lee and Li, 1989). These mechanisms are still poorly understood (Lee and Li, 1989).

There are few experiments on non-corneal drug absorption in the literature. Ahmed and Patton (1985) showed that the conjunctival/scleral permeation was the major route for inulin absorption to the iris and ciliary body. Chien et al. (1990) demonstrated that it was a major route also for the absorption of hydrophilic p-aminoclodine. Hayakawa et al. (1992) evaluated the conjunctival permeation of hydrophilic atenolol (mw 266), lipophilic timolol (mw 433) and insulin (~ mw 5700). The conjunctival permeability of insulin (P_{app} 0.46 x 10^{-5} cm/s) was about two times lower than that of atenolol (P_{app} 0.95 x 10^{-5} cm/s) and five times lower than that of timolol (P_{app} 2.21 x 10^{-5} cm/s). Sasaki et al. (1995a) noticed that the conjunctival permeability to beta-blockers with or without absorption promoters was greater than the corneal permeability. Ahmed et al. (1987) showed with several permeants that the conjunctiva of the rabbit was more permeable than its cornea.

Olsen et al. (1995) determined the *in vitro* permeability of human sclera to hydrophilic compounds varying in molecular weight (mw range 130- 70 000). They noticed an inverse relationship between the scleral permeability (P_{app} range 1.9 x 10^{-6}- 44.0 x 10^{-6} cm/s) and molecular weight. Prausnitz and Noonan (1998) concluded on the basis of their database collection of scleral permeability measurements that the permeability of sclera (P_{app} range 1.3 x 10^{-7} – 7.1 x 10^{-5} cm/s) has no apparent dependence on PC (logPC range was –3.72 – 4.04) and a strong dependence on molecular radius (at radii of 0.1- 6.4 nm). Ahmed et al. (1987) claimed that the solute size had a more pronounced effect on permeability in the sclera than in the cornea. The model compounds were penbutolol (mw 291), propranolol (mw 259), nadolol (mw 309), timolol (mw 316), sucrose (mw 342) and inulin (mw 5000). The inulin permeability was compared to sucrose permeability. In the sclera P_{app} of inulin was 16 times and in the cornea 8 times lower than P_{app} of sucrose. Kao et al. (1990) found that the sclera was 11 times more permeable to a topical carbonic anhydrase inhibitor than the cornea.

Conjunctival and scleral permeation have been found to be less sensitive to the changes in the lipophilicity of penetrant than the corneal permeation (Ashton et al., 1991; Sasaki et al., 1995a; Wang et al., 1991). For example, with β-blockers (logPC range –0.62-3.44), there was an 8 fold difference in conjunctival P_{app} as compared with a 48 fold difference in the values of corneal P_{app} (Wang et al., 1991). Conjunctival P_{app} range was from 2.4 x 10^{-5} to 6.2 x 10^{-5} cm/s and corneal P_{app} range was from 0.1 x 10^{-5} to 3.2 x 10^{-5} cm/s. Thus, the difference appears to be most obvious in the case of hydrophilic drugs.

The results on the relative permeabilities of the compounds are somewhat conflicting. Conjunctiva has been shown to represent a more permeable membrane for hydrophilic drugs than the cornea and sclera (Sasaki et al., 1995a, b; 1997). The scleral permeability for hydrophilic drugs has also been reported to be higher than the corneal permeability (Sasaki et al, 1995b; Ahmed et al., 1987), but sclera was less permeable to lipophilic drugs than cornea (Sasaki et al., 1995b).

2.1.3 Ocular retention

The typical volumes of commercial eyedrops are 25-56 µl (Lederer and Harold, 1986) while normal tear volume is only 7 µl. When eyedrops are instilled to the ocular surface the solution is diluted with the tear fluid and extra solution flows rapidly from the ocular surface through the puncta to the lacrimal drainage system and, then, to the nose, pharynx and GI-tract (Chrai et al., 1974). Sometimes, part of the instilled solution spill over to the cheeks. Consequently, the ocular contact of the solution and the time available for drug absorption are short. For instilled eyedrop solutions, typical corneal contact times are about 1 to 2 minutes in humans, and ocular bioavailability is usually less than 5% (Robinson, 1989). The drainage rate of the instilled eyedrop is rapid; from an ocular surface it is typically 0.5-0.7 min^{-1} in rabbits and 1.5 min^{-1} in humans (Lee and Robinson, 1986). This difference may be due to higher blinking frequency in humans (Saettone et al., 1982; Zaki et al., 1986). The larger the eyedrop volume, the faster the drainage rate: for 5 µl it is 0.31 min^{-1}, for 50 µl it is 0.82 min^{-1} in rabbits (Chrai et al., 1973). The normal rate of tear turnover is approx. 0.16 min^{-1} (1.2 µl/min) (Sugrue,

1989) in humans and 0.07 min^{-1} (0.5 µl/min) in rabbits (Urtti et al., 1990) and, accordingly, it has a minor role in the removal of the instilled solution from the ocular surface.

A substantial decrease in the eyedrop volume can maximally improve the ocular bioavailability of a hydrophilic drug by four fold, while systemic drug absorption is decreased (Keister et al., 1991). In the case of a drug with high permeability, decreased eyedrop volume does not increase the ocular bioavailability (Keister et al., 1991). Increasing the viscosity of the eyedrop decreases the drainage (Zaki et al., 1986) and it was suggested that a minimum viscosity of 20 centistokes is needed to enhance corneal drug absorption. The acidic pH, hypertonicity, and hypotonicity of the eyedrop can increase the lacrimation and, thereby, decrease ocular drug absorption (Conradi et al., 1978).

Often more than 50 % of ocularly instilled drug dose is absorbed systemically through the conjunctiva and nasal mucosa (Urtti and Salminen, 1993). Conjunctival uptake of a topically applied drug from the tear fluid is typically an order of magnitude greater than its corneal uptake (Urtti et al., 1985; Thombre and Himmelstein, 1984).

Due to the short ocular contact time new improved dosage forms have been studied. Several approaches have been tried to prolong the ocular contact time. Some of the topical ocular controlled release systems tried include suspensions, liposomes, polymer matrices and reservoir devices (Le Bourlais et al., 1995). The dosing interval of the drug can be prolonged in this way. The retention of the dosage form in the conjunctival sac and the *in vivo* release rate of the drug are the main factors that determine the drug input to the tear fluid after application of the system (Urtti, 1995). Poor retention in the preocular area may lead to the removal of the dosage form from the conjunctival sac before the drug is released (e.g. liposomes, nanoparticles). A reservoir device that releases pilocarpine for a week has been in clinical use to treat glaucoma (e.g.Ocusert®) (Sihvola and Puustjärvi, 1980). Also, pilocarpine and timolol gels and betaxolol microparticles are used in the treatment of glaucoma.

It has been shown that the controlled release ocular systems enhance the absorption of drugs into the aqueous humour as compared to eyedrops (Finne et al., 1990, Urtti et al., 1990). When timolol was present in the ethylene vinyl acetate matrices, it was

absorbed better to the iris-ciliary body and aqueous humour than from eyedrops (Friedrich et al., 1996). The authors suggested that this was likely due to an increase in the amount of timolol, which entered the interior of the eye through the non-corneal permeation. Thus, polymeric controlled release systems target drug absorption to the conjunctival and scleral route. This may be advantageous in the case of biotechnologically produced large drug molecules (i.e. peptides, oligonucleotides) (Krishnamoorthy and Mitra, 1993).

2.2 Epithelial transport

Drug absorption, distribution, and elimination are governed by their permeation across different biomembranes in the body. Basic mechanisms of transport are similar in different epithelia. Physiological factors and the properties of the drug, which may affect the transport, are listed in Table 1 (Fischer Weiss, 1996).

Table 1. Factors influencing drug transport in epithelia.

Physiological factors	Drug properties
Lipid bilayer composition	size
Surface area	charge
Metabolism	lipophilicity
Binding	conformation
Blood flow	hydrogen-bonding capacity
Tight junctions	
Thickness of epithelium	
Efflux systems	
Active transport	

2.2.1 Transcellular permeation

Most ophthalmic drugs in clinical use permeate transcellularly through anterior ocular membranes. The transcellular route consists of two barriers, apical and basolateral cell membranes (Powell, 1981)(Fig. 3). The tight junction participates in the polarization of the epithelial plasma membrane into the compositionally distinct apical and basolateral domains and it is believed to function as a "fence" in the plasma membrane (Gumbiner, 1987). Drugs may permeate transcellularly by passive or active mechanism (Fischer Weiss, 1996).

In passive transcellular diffusion, drugs permeate by a partition-controlled process (Grass and Robinson, 1988a; Lee, 1990). Partitioning allows equilibration of drug into the lipid bilayers of the epithelial membrane. In addition to partitioning, drug diffusion through the lipid bilayer is crucial in transcellular permeation. This sets limitations to the molecular size and shape that can fit into the lipid bilayers (Fischer Weiss, 1996).

For large hydrophilic drugs which do not partition well into the cell membrane (some peptides and proteins) transcellular endocytotic fluid-phase pinocytosis may be one of the transport routes (Rojanasakul et al., 1992).

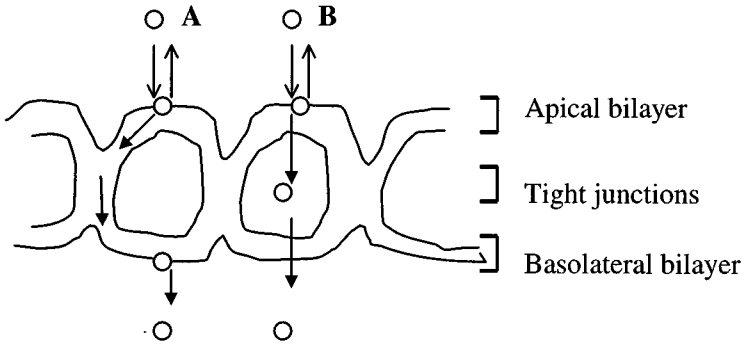

Figure 3. Potential mechanisms for passive transcellular diffusion of a solute. **A**: the solute adsorbs to the cell membrane surface and diffuses laterally, within the membrane, to the basal side. **B**: the solute diffuses across the cell membrane and enters the cytoplasm before exiting the cell across the basolateral membrane (Burton et al., 1991).

Ions can be actively transported across the corneal epithelium and endothelium. Active ion transport mechanisms are essential for maintaining the normal stromal hydration and, corneal shape and transparency. The corneal epithelium contains ionic channels that are selective for cations (Rae, 1993) and it also contains an outwardly rectifying anion channel in the apical membrane and a highly conductive potassium (K^+) channel in the basal cells. Sodium (Na^+) and chloride (Cl^-) are most actively transported ions in the corneal epithelium. Na^+ permeates from the tears into the epithelium by passive diffusion, but it is actively transported from the epithelium to the stroma. Cl^- is actively transported from the stroma into tears (Klyce and Crosson, 1985). Na^+-K^+-ATPase is involved in the transport of Na^+ and Cl^-. Ion transport pumps may have a role in the active transport of peptides (Klyce and Crosson, 1985).

The corneal endothelium contains also Na^+ and K^+ selective channels and ionic channels that are selective for cations (Rae, 1993). In addition, Ca^{2+} channels are thought to be present in the plasma membranes of endothelial cells (Green et al., 1994). Specific ions are actively transported from the stroma to the aqueous humor by the endothelium (Green et al., 1994).

In the conjunctiva, it has been demonstrated that Na^+ (Shi and Candia, 1995) and Cl^- (Kompella et al., 1993) and some drugs are actively transported. Na^+-amino acid cotransport has been found to be one of the mechanisms for mucosal amino to enter into epithelial cells of the pigmented rabbit conjunctiva (Kompella et al., 1995; Horibe et al., 1997; Hosoya et al., 1997). Also, Na^+-dependent and Na^+-independent nucleoside transporters are localized on the mucosal side of the pigmented rabbit conjunctiva (Hosoya et al., 1998). Basu et al. (1998) noticed that uptake of the dipeptide L-carnosine in primary cultured rabbit conjunctival epithelial cells is mediated by a proton-driven dipeptide transporter. Therefore, the role of active transporters may be more important in ocular pharmacokinetics than was previously believed (Basu et al., 1998).

In the rabbit conjunctiva, there may exist a p-glycoprotein-mediated drug efflux pump on the apical side to restrict the absorption of cyclosporin A and other lipophilic drugs (Saha et al., 1998).

2.2.2 Paracellular permeation

The aqueous intercellular space forms the paracellular route of drug permeation across the epithelia. It consists of aqueous pores among the strands of the cellular zonula occludens ZO; (tight junctions) (Fig. 4 and 5) (Balda and Matter1998; Balda et al., 1996; Claude, 1978). The current model of tight junction is B in Fig. 5. Other members of the junctional complex include zonula adherens, the Ca^{2+}-dependent cell adhesion molecule uvomorulin, and desmosomes (Gumbiner, 1987). The ZO is one of the major hallmarks of absorptive and secretory epithelia. It controls the diffusion of ions and neutral molecules through the paracellular route (Gumbiner, 1987). The seal provided by ZO does not appear to constitute an absolute diffusion barrier since it allows the passage of water and certain solutes (Grass and Robinson, 1984; Cereijido et al., 1993). In MCDK cell epithelium, it has been reported that water does not flow convectively

across the tight junctions (Kovbasnjuk et al., 1998). Several intracellular proteins (e.g. cadherins, ZO-1, ZO-2, cingulin) control the tight junctions (Citi et al. 1988; Lutz and Siahaan, 1997). The sealing, an intercellular protein was found by Furuse et al. (1993) and it was named occludin. The level of occludin expression determines the transepithelial resistance (TER) (Balda et al., 1996) and, therefore, the paracellular pores may represent the open space between occludin strands (Fig. 4). The integrity of epithelial ZO is known to depend e.g. on extracellular Ca^{2+} (Gumbiner, 1987).

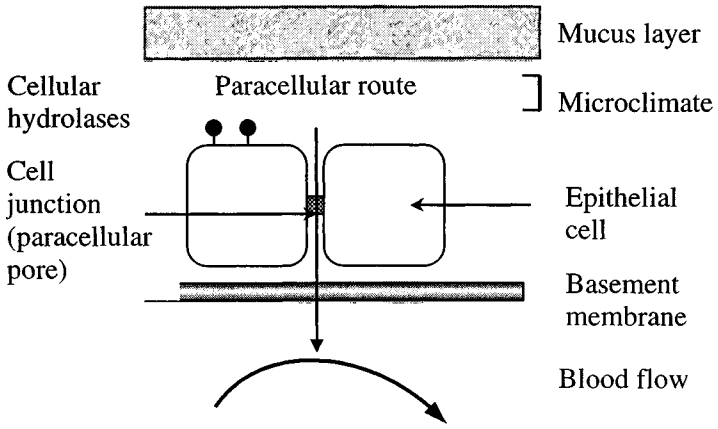

Figure 4. The paracellular route (Mackay et al., 1991).

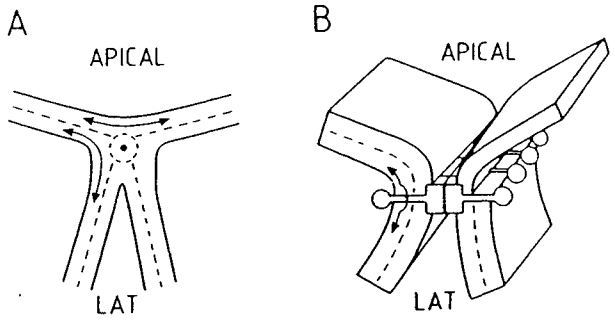

Figure 5. The models for structure of tight junction (Gumbiner 1987). A: fusion model in which plasma membranes of adjacent cells merge. B: model in which integral membrane proteins are as major structural elements of the tight junction.

Paracellular permeation and pore size. The paracellular surface area is small compared to the transcellular surface area. Hydrophilic compounds permeate through paracellular route, this being the primary route of passive ion permeation. The charge and molecular size affect the paracellular permeation (Banks and Kastin, 1987).

Tonjum (1974) found that the outermost cell layers of the corneal epithelium were impermeable to horseradish peroxidase, indicating that the paracellular pores of the cornea were smaller than 3.0 nm. Grass and Robinson (1988b) estimated that the limiting size of a molecule undergoing paracellular permeation through the cornea is 3.0 nm or less. It has also been reported that the limiting size of the corneal paracellular pores would be 1.2 nm, the molecular diameter of glycerol (Grass and Robinson, 1988b, c), or 3.0 nm, the molecular diameter of inulin (Lee et al., 1986b). For comparison, the aqueous pores in the nasal epithelium have been reported to be 0.4-0.8 nm; in the jejunum, 0.7-1.6 nm; in the rectum, 0.6-1.7 nm (Hayashi et al., 1985), and in the alveolar epithelium two kinds of pores; 0.6-1.0 nm and large pores 7.0-12.0 nm (Taylor and Gaar, 1970). In the small intestine, the average size of the aqueous pores was estimated to be 0.7-0.9 nm. Madara and Darmsathaphorn (1985) reported that ZO completely occludes intestinal diffusion of molecules with molecular radii larger than 1.1 nm.

Paracellular permeability and electrical resistance. The number of tight junction strands in the ZO seems to be the most important factor of the paracellular electrical resistance of various epithelia (Balda et al., 1996). Epithelia (such as renal proximal) with electrically leaky tight junctions (appr. 5 Ω cm^2) might only have a single continuous strand circumscribing the cell apex. A very tight epithelium (such as that present in toad urinary bladder)(several thousand Ω cm^2) could have up to eight strands lying in parallel, covering the ZO from the apical to the basolateral membrane (Claude, 1978). It is still not clear whether tight junctions are due to transmembrane proteins or specialized lipid structures. However, it has been noticed that electrical resistance does not always correlate with the number of strands (Stevenson et al., 1988). The electrical resistance depends on how well neighbouring plasma membranes are interconnected as well as on the conformation of pores (open or closed)(Balda et al., 1996).

Rojanasakul et al. (1992) measured the membrane resistance in various epithelia in the rabbit. The highest membrane resistance values were in skin (9703 Ω cm^2), buccal (1803 Ω cm^2), and corneal epithelia (1012 Ω cm^2) and the lowest in the intestinal ($\approx$ 200-300 Ω cm^2). The electrical resistance is related to the dimensions of the pores, to the tortuosity of the intercellular space and also to the permeability of the epithelia (Claude, 1978). However, paracellular permeability does not always correlate with the electrical resistance (Balda et al, 1996). The paracellular permeability depends on the number of open paracellular pores per barrier (Balda et al., 1996). This was shown in cultured renal epithelium (MDCK) cells.

2.3 Models to estimate paracellular pores in biomembranes

Renkin (1955) presented for steric hindrance of the pore entrance using the following equation:

$$A_{sd}/A_p = (1-\alpha)^2 (g^o/g) \quad (1)$$

Where A_{sd} is the apparent area for diffusion, A_p is the total pore area, α= a/r, in which a is the radius of the solute molecule and r is the pore radius.

$$g^o/g = 1 - 2.104\alpha + 2.09\alpha^3 - 0.95\alpha^5 \quad (2)$$

Equation 1 is applicable if α is not greater than 0.4. In other words, the radius of the solute should be much smaller than the pore radius. In these circumstances, the basic assumption, random walk of diffusion ('drunken sailor type'), is valid.

Renkin also derived an equation for bulk flow through the pores and assumed that laminar flow took place through the membrane and that the velocity of the flow in each laminar shell depends on the distance of the shell from the axis of the tube. Renkin gave the following equation for the frictional effects in bulk flow:

$$A_{sf}/A_p = [\, 2(1 - \alpha)^2 - (1-\alpha)^4] \, (g^o/g) \quad (3)$$

Where A_{sf} in the apparent area for filtration. In general, it is not possible to measure A_p in biological membranes, so water flow was measured, and the ratio A_{sf}/A_{wf} is used.

$$A_{sf}/A_{wf} = \frac{[2(1 - \alpha_s)^2 - (1-\alpha_s)^4] \quad (g_s^o/g_s)}{[2(1 - \alpha_w)^2 - (1-\alpha_w)^4](g_w^o/g_w)} \qquad (4)$$

Where the A_{wf} is the apparent area for filtration of water. The subscript s refers to use of the solute radius, and w refers to the water radius.

Paganelli and Solomon (1957) introduced the term equivalent pore radius to describe the operational nature of the description: a radius equivalent to the pore radius of an ideal membrane which contains uniform, circular pores in which diffusion and bulk flow may be described by Fick's and Poiseuille's equations. In modeling it is usually assumed that the epithelial ZO pores are cylindrical and have equivalent radius (Horibe et al., 1997)

Fick's law:

$$J_s = - D_s (\delta c/\delta x) \qquad (5)$$

Where
J_s = solute flux (mol/scm^2),
c = concentration (mol/cm^3),
x = distance (cm),
D_s = diffusion coefficient in solution (cm^2/s)

Poiseuille's flow:

$$V = 2V_0(1 - \beta^2) \qquad (6)$$

Where
β = dimensionless radial position r/r_0 of the center of the solute molecule,
V_0 = average velocity as defined by the total volume flow rate through one pore (solvent + solute) divided by the pore cross-section

Pappenheimer et al. (1951) pointed out that the ratio of the hydraulic conductivity, measured under either an osmotic or a hydraulic pressure gradient, to the transmembrane water-diffusion coefficient, as measured by tracers, provides data which can be used to calculate an equivalent pore radius for the channels in the membrane. Pappenheimer et al. (1951) determined with equation 7 the equivalent pore radius (3.0

nm) in capillary membranes. If the only substance that traversed through the pores is water:

$$J_v = (A_w/\Delta x)(r^2/8\,\eta_w)\Delta P \qquad (7)$$

Where η_w is the viscosity of water, ΔP is the pressure difference, A_w the restrictions to diffusion by the membrane (tracer water), solution (cm^2/s), Δx is the total path length through the membrane, and r is the pore radius.

Solomon (1959) gave the following equations for the calculation of the equivalent pore radius:

$$\lambda = (8\eta_w D_w/k')(P_f/P_d - 1) \qquad (8)$$

Where λ is related to r by equation (9) below. D_w is the diffusion coefficient for water, and k' is a conversion factor having the value of 1.35×10^9 dynes/cm^2 at 23°C. At this temperature $(8\eta_w D_w/k') = 14.3 \times 10^{-16}$ cm^2. P_f and P_d are permeability coefficients for filtration and diffusion, respectively. The difference between steric hindrance in diffusion and bulk flow should be taken into account.

$$r = -a_w + \sqrt{2a_w^2 + \lambda} \qquad (9)$$

Where a_w is the radius of the water molecule.

From the diffusivities of several solutes, Solomon (1959) calculated an equivalent pore radius of 0.35-0.45 nm for erythrocyte membranes. Similarly Stein (1967) showed that several other biological barriers have equivalent pore radii between 0.4 and 0.6 nm.

Lonquet-Higgins and Austin (1966) concluded that for equivalent pore radii less than 0.45 nm, a diffusional mechanism would be operative; above this value, the mechanism of transport would be hydrodynamic, by Poiseuille flow.

In theory, when the pores of the membrane are so narrow that water diffuses through the pore, the flux is proportional to the pore area (r^2). When the pores are so large that

the diffusion is negligible compared to viscous flow, Poiseuille's law obtains and flux is proportional to r^4 (Solomon, 1968).

The validity of the equivalent radius calculations is strictly dependent on the assumption that all solutes permeate only through aqueous pores (Flynn et al., 1974). In addition, it is important to estimate or know the dimensions of the permeant. Stokes-Einstein equation has generally been used to approximate the molecular radius for a solute (Anderson and Quinn, 1974; Davidson and Deen, 1988; Ruddy and Hadzija, 1992; Adson et al., 1994):

$$D_\infty = \frac{kT}{6\pi\eta r_s} \qquad (10)$$

In Eqn. 10, η is the viscosity of the solvent (poise), r_s is the Stokes-Einstein radius (cm) of solute molecule, T is the absolute temperature, k is the Boltzmann's constant, and D_∞ is the solute diffusivity in dilute bulk solution. In this case the shape of the molecules is assumed to be spherical

The Renkin equation (1) for cylindrical pores has been used to calculate the junctional pores in a human colorectal carcinoma cell line (Caco-2) (Conradi et al., 1991; Adson et al., 1994; Knipp et al., 1997), MDCK, and alveolar epithelial cell monolayers (Adson et al., 1994). Conradi et al. (1991) estimated for the zwitterionic peptides (molecular radius range 0.45-0.6 nm) that the pore size of Caco-2 cell monolayers was approx. 0.8 nm. Adson et al. (1994) estimated the effective pore sizes in cultured cell monolayers using hydrophilic compounds varying in molecular radii (range 0.27- 0.6 nm) and charge. The results showed that the effective pore radius in Caco-2 cell monolayer was 1.2 nm, in MDCK cell monolayer it was 1.1 nm and in alveolar cell monolayer it was 0.55 nm (Adson et al., 1994). Knipp et al. (1997) estimated with hydrophilic compounds with different charge and size (molecular radii range 0.25-0.48 nm) that the pore size in unperturbed Caco-2 cell monolayer was 0.52 nm whereas in a maximal perturbed monolayer (compounds with enhancers) it was 1.56 nm.

2.4 Ocular peptide and protein absorption

The potentially useful peptides and proteins in ophthalmology include cyclosporine, interferons, enkephalins, various growth factors and hormones for uveitis, wound healing, herpes simplex infections, pains and modification of immune response (Harris et al., 1992). In addition, integrin-binding RGD-peptides may inhibit ocular neovascularization (Hammes et al., 1996) and atriopeptin is able to lower intraocular pressure (Korenfeld and Becker, 1989; Wolfensberger et al., 1994).

The molecular size, charge, and hydrophilicity of the peptides and proteins affect their permeation through the corneal and conjunctival epithelium (Banks and Kastin, 1987). Most peptides and proteins are hydrophilic and, thus, the pore radius of the paracellular route of the epithelium limits the permeation of the peptides.

In addition to the physical barrier, the enzymatic barrier of the membranes is an important determinant of the ocular bioavailability of peptides and proteins (Pert et al, 1976; Stratford and Lee, 1985). Peptidase activity in the anterior membranes of the eye and in the tear fluid has been reported to be sufficient to hydrolyze labile peptides (Lee et al., 1986a). Aminopeptidases that cleave peptides and proteins at the N-terminus amide bond are the most active peptidases in the eye (Lee et al., 1986a).

Ocular absorption of enkephalins, pentapeptides, have been investigated previously. Lee et al. (1986a) found that topically applied enkephalins absorbed in small amounts into the albino rabbit eye, but they were also degraded to different extents in the corneal epithelium and other anterior membranes. The percentage of intraocular absorption of peptide derived total radioactivity was 0.9% for leucine enkephalin, 0.6% for methionine enkephalin, and 0.1% for [D-Ala^2]-Met-enkephalinamide (DAMEA). Only a fraction of that radioactivity represented intact peptide at 5 min post-dosing (13% of leucine enkephalin, 1% of methionine enkephalin, and 74% of DAMEA). Pre-treatment with an aminopeptidase inhibitor, bestatin did not improve the bioavailability. Dodda Kashi and Lee (1986) found that DAMEA was 11-23 time more stable to hydrolysis in the corneal epithelium, iris-ciliary body, conjunctiva, and tears and 2-5 times more stable to hydrolysis in the corneal stroma than leucine enkephalin and methionine

enkephalin. They also reported that both aminopeptidases and dipeptidyl peptidase and dipeptidyl carboxylpeptidase degraded these peptides to a varying extent.

Several attempts to modify and increase peptide absorption have been published. The protease inhibitors (e.g. bestatin, puromycin) have been coadministered with peptides with variable success (Lee et al., 1986a; Stratford and Lee, 1985). Metabolically resistant peptides and prodrugs have been developed to enhance the peptide absorption (Pert et al., 1976; Gudmunsson et al., 1999a, b). The cytoskeletal modulator, cytochalasin B has been reported to be able to induce similar increase in the corneal permeability, but with substantially less membrane damage, when compared to conventional permeation enhancers such as bile salts and ethylenediaminetetraacetic acid (EDTA).

Some investigators have suggested the use transconjunctival route to deliver peptides and proteins systemically. These attempts have had only limited success (Chiou et al., 1988; Yamamoto et al., 1989). Chiou et al. (1988) reported that the maximum amount of peptide drugs that can be delivered through the conjunctiva is limited to 5mg and the maximum molecular weight of peptide can be 10 000. Stratford et al. (1988) found that systemic absorption of DAMEA was similar to that of epinephrine and approx.10 times greater than that of inulin. Compared to other routes of systemic peptide administration (pulmonary, nasal, transdermal), the ocular route may not be good alternative.

2.5 Polymer matrix systems for ophthalmic drug delivery

Absorption of drugs into the eye can be improved by good corneal or non-corneal permeation and prolonged contact time with the ocular surface. One way to prolong the preocular drug retention is to use erodible or nonerodible polymer matrices (Le Bourlais et al., 1995). Usually the polymer matrices are easily made by solvent casting or by compression molding. The matrices can either dissolve in the tear fluid or they can be insoluble. In the latter case, the matrix must be removed when the entire drug has been released. For ocular drug delivery, erodible and water-soluble matrices are preferred, because they need not be removed after the dosing period (Le Bourlais et al., 1995).

The drug release mechanism can be physical or chemical in nature, but diffusion is always involved (Leong and Langer, 1987). In a physically controlled release system,

the release may be controlled by drug diffusion or solvent penetration into the matrix. For a nonerodible matrix, release is by diffusion and it is driven by a concentration gradient. It can also be driven by matrix swelling (solvent-activated systems). The release of drug depends on the relaxation of the polymer as a result of water absorption (Hopfenberg and Hsu, 1978; Peppas and Korsmeyer, 1986). In an ideal swelling-controlled drug delivery system, the velocity of the moving waterfront that triggers polymer relaxation, and, subsequently, allows drug diffusion, controls drug release (Klech and Li, 1990). Often release is partly controlled by the rate of polymer relaxation.

The chemically controlled system can be for example biodegradable or bioerodible matrix in which the drug is dispersed or dissolved. The dissolution of the matrix is caused by the hydrolytic or enzymatic cleavage of the backbone of the polymer or conversion of the polymer to a soluble form (Langer and Leon, 1987; Smith et al., 1990). If solely the degradation or erosion of the matrix controls the drug release, a constant zero-order release can be obtained, provided that the surface area of the matrix remains constant. This is difficult to achieve and often diffusion of the reactants and the drug molecules may still be rate limiting. Thus, the release may be an intermediate between erosion control (zero-order) and diffusion control (square root of time kinetics).

In general solute release from the controlled release polymer devices is described by equation:

$$M_t/M_\infty = kt^n \qquad (11)$$

Where M_t/M_∞ is the fractional solute release, t is the release time, k is a constant, and n is the exponent characteristic of the release mechanism (Ritger and Peppas, 1987a, b). Table 2 shows the relationship of the diffusional exponent and the mechanism of diffusional release from a thin slab-shaped non-swellable and swellable controlled release systems.

Table 2. Diffusional exponent and mechanism of diffusional release from thin non-swellable and swellable controlled release systems.

Controlled release system	Diffusional exponent n	Drug release mechanism
Non-swellable	0.5	Fickian diffusion
	0.5-1.0	Anomalous (non-Fickian) transport
	1.0	Zero-order release
Swellable	0.5	Fickian diffusion
	0.5-1.0	Anomalous (non-Fickian) transport
	1.0	Case-II transport

When the permeation of water into the matrix is rapid compared to the drug diffusion, the drug is released according to the Fickian diffusion (Lapidus and Lordi, 1968). Anomalous diffusion is observed when the rates of solvent permeation and drug release are in the same range (Korsmeyer and Peppas, 1981, 1983). When the rate of drug diffusion controlled the release from the polymer matrices, zero-order drug release can be achieved by having a special geometry in the device (Hsieh et al., 1983) or suitable non-uniform distribution of the drug in the matrix (Lee, 1984).

Concentration, surface area, leaching solvent, and polymer system (Borodkin and Tucker, 1974) may control the release rate of drugs from polymer matrix. In a diffusion-controlled system, the physicochemical properties of drug may also limit the release rate. For example, the molecular weight of the diffusing molecule affects its diffusivity in the polymer network (Burchak et al., 1994). An erodible system is less dependent on the properties of the drugs.

The objective of this section is to present the earlier studies, which have been, carried out with bioerodible PVM/MA matrices and by Gelfoam®, which is a hydrogel forming delivery system.

2.5.1 Poly(vinyl methyl ether/maleic anhydride) (PVM/MA) polymer

PVM/MA polymers contain two essential components, which permit drug release and erosion: a solubilizing carboxylic group and a hydrophobic ester group that decreases the permeation of water into the polymer. The degree of ionization of the carboxylic acid group, the pH of the surrounding medium and the pK_a of the carboxylic

group affect the dissolution rate of the polymers (Heller et al., 1978; Heller and Trescony, 1979). In addition, the ionization of the carboxylic groups in the polymer decreases the microclimate pH on the polymer surface, especially, if the buffering capacity of the medium is weak. This phenomenon has been explained to be due to the accumulation of hydrogen ions that dissociate from the carboxylic acid groups on the polymer surface. This blocks further ionization and polymer dissolution. The apparent pK_a of the monoisopropyl ester of PVM/MA has been determined to be 5.3 (Finne et al., 1989).

Earlier PVM/MA studies have been carried out with conventional small molecular weight ophthalmic drugs. Heller et al. (1978) studied the release of poorly hydrophilic drug hydrocortisone from the cast matrices of alkyl monoesters of PVM/MA. Hydrocortisone released at a constant rate from n-butyl (Heller et al., 1978) and from n-hexyl (Heller and Trescony, 1979) monoesters of PVM/MA. The erosion of the matrix surface controlled the rate of hydrocortisone. Urtti (1985) studied the release of hydrophilic pilocarpine from the half-esters of PVM/MA. The release was not controlled by erosion; part of the pilocarpine diffused from the uneroded part of the matrices, but nonetheless zero-order release was achieved, presumably due to case II transport. The increasing buffer concentration in the dissolution medium increased the rates of pilocarpine release and polymer dissolution (Urtti, 1985).

Timolol release from the monoisopropyl esters of PVM/MA has been studied extensively by Finne et al. (1989-1992). Timolol maleate, timolol and hydrocortisone released from the matrices of monoisopropyl ester of PVM/MA according to the zero-order kinetics in 10-160 mM phosphate buffer solutions (Finne et al., 1989). The release rate of timolol was increased both *in vitro* and *in vivo* in rabbit eyes when basic salts (disodium phosphate, sodium acetate, trisodium citrate and borax) were added to the matrices. This was shown by measurements of drug concentrations in the tear fluid, ocular tissues and plasma. The aqueous humor/plasma concentration ratio was increased with polymer matrices, the best result, almost 20-fold improvement, was seen by applying the timolol in acetate buffered matrices (Finne et al., 1991).

Drug release *in vivo* from polymeric systems in rabbit eyes was found to be similar (Urtti et al., 1994) or different to (Finne and Urtti, 1992) *in vitro* release. The monoester

of PVM/MA released timolol at lower rates *in vivo* in rabbit eyes than *in vitro* in 40 mM phosphate buffer. This release was equal at 2 mM phosphate buffer. The polymer dissolution is dependent on surface pH and the transport of hydrogen ions across the diffusion layer on the polymer surface (Heller et al., 1978). The thickness of the diffusion layer was increased *in vivo* because of the low buffering capacity of the tear fluid and poor mixing. Subsequently, the low local surface pH at the polymer surface could not maintain the fast dissolution. Consequently, the rates of polymer dissolution and timolol release were lower *in vivo* than *in vitro* (Finne and Urtti, 1992).

Earlier studies have demonstrated that monoesters of PVM/MA deliver drugs into the eye with advantageous kinetics. Erosion controlled kinetics may be applicable also to the release of large molecules. Therefore, these polymers may feasibly used for ocular delivery of peptides via non-corneal route.

2.5.2 Gelfoam®

Gelfoam® is an absorbable gelatin sponge that is used on bleeding surfaces as a hemostatic (Gelfoam brand, 1991). The polymer swells but it does not dissolve in water. It is prepared from purified pork skin Gelatin USP granules by a thermal treatment method. The advantages of Gelfoam® include its non-allergenic nature, low price and biodegradability. The absorbable gelatin device will not have to be removed from the conjunctival sac at the end of the drug-dosing period.

Due to rapid systemic drug absorption after topical ocular application some researchers have utilized ocular application to deliberately deliver peptides into the systemic circulation. Nadkarni et al. (1993) studied pilocarpine release from Gelfoam devices, without any additivies and also devices, which were embedded with PEG 400-monostearate or cetyl ester wax. The Gelfoam device released all of its pilocarpine in 15 minutes, while the device embedded with cetyl ester wax released pilocarpine according to a zero-order kinetics during 5 h but the devices with PEG 400-monostearate exhibited anomalous (non-zero order) drug release. Simamora et al. (1996) studied the systemic delivery of insulin with polyoxyethylene-20-stearyl ether (Brij-78) from the Gelfoam device *in vivo* in rabbits. With the Gelfoam device it was possible to prolong and intensify the effect of insulin on blood glucose levels in rabbits. Lee et al. (1997 a, b)

studied also the systemic delivery of insulin after ocular application to rabbits without enhancers and with Brij-78 in the Gelfoam device. Brij-78 enhanced the systemic insulin delivery. *In vivo* data in rabbits showed that melanotan II (a cyclic heptapeptide) had a 67% systemic bioavailability if it was delivered by Gelfoam device compared to 25 % after eyedrop application (Pinsuwan et al., 1997).

The Gelfoam devices have been well tolerated; the only adverse effect has been blinking immediately after the dry device has been instilled to the cul-de-sac. The blinking stopped within a few seconds when the device became hydrated (Lee et al., 1997 a; Pinsuwan et al., 1997). Due to its previous clinical use as an absorbable medical device, Gelfoam® should be well tolerated and safe for the local and systemic administration of different drugs. However, the drug-releasing characteristics from Gelfoam® have not been studied systemically.

3 AIMS OF THE STUDY

The main purpose of this work was to increase understanding about the ocular delivery of hydrophilic molecules in terms of their ocular permeation and release from polymeric inserts. The specific aims were:

1. To compare the molecular size dependence of the corneal, conjunctival and scleral permeation using hydrophilic polyethylene glycols as model compounds *in vitro*.

2. To develop a new approach for quantitative determination of the paracellular porosity and pore size in epithelia.

3. To determine the physical and enzymatic barriers preventing corneal, conjunctival and scleral permeability of D- [Ala^2]-methionine enkephalinamide (DAMEA).

4. To study the combined effects of an aminopeptidase inhibitor and an enkephalinase inhibitor on the corneal, conjunctival and scleral permeability of DAMEA *in vitro*.

5. To study the effects of the molecular weight, charge, matrix buffering, and drug content on drug release from the polymer matrices made of monoisopropyl ester of PVM/MA and Gelfoam.

4 MATERIALS AND METHODS

4.1 *In vitro* permeability studies (I, II, III, IV)

4.1.1 Animals

Male and female albino New Zealand rabbits weighing between 3.0-4.5 kg (**I, II, III**) and 2.2-3.6 kg (**IV**) were used as experimental animals. The rabbits were housed in standard laboratory rabbit cages and fed regular diet with no restrictions on the amount of food or water consumed. All *in vitro* permeability tests conformed to the ARVO Resolution on the Use of Animals in Research.

4.1.2 Model compounds and solutions

PEGs with mean molecular weights of 200 (M_w/M_n = 1.11), 400 (M_w/M_n = 1.07), 600 (M_w/M_n = 1.10) and 1000 (M_w/M_n = 1.05) were obtained from Chemical Pressure Co. (Pittsburgh, PA) (**I, II, III**). Polypropylene glycol (PPG) 425 (Aldrich, Steinheim, Germany) was used as an internal standard (**I, II, III**).

The PEG-solution was made by weighing first PEG 200 (final concentration 2.0 mg/ml), PEG 400 (4.0 mg/ml), PEG 600 (6.0 mg/ml) and PEG 1000 (10.0 mg/ml) and dissolving in glutathione Ringer's solution (GBR)(O'Brien and Edelhauser, 1977) (**I, II, III**). The standards were prepared by diluting PEG-solution with GBR. The dilutions corresponded to 0.002, 0.05, 0.1, 0.3 and 1.0% transmembrane permeation of PEG.

[D-ala^2]-methionine enkephalinamide (DAMEA), bestatin and standard amino acids and peptides were obtained from Sigma Chemical Co. (St.Louis, MO, USA) (**IV**), except Tyr-D-Ala (Haartman Institute, University of Helsinki, Helsinki, Finland), and Tyr-D-Ala-Gly-Phe (Bachem, Budendorf, Switzerland). The neutral endopeptidase inhibitor SCH 39370 was a kind gift from Schering-Plough (Kenilworth, NJ, USA).

DAMEA (final concentration 1.0 mM) was weighed and dissolved in GBR (O'Brien and Edelhauser, 1977) (**IV**). When the inhibitors were present, SCH 39370 was weighed (final concentration 0.25 mM) and dissolved in GBR and, then, DAMEA and bestatin (final concentration 0.25 mM) were added. The standard mixtures containing DAMEA,

Tyr, Tyr-D-Ala-Gly were prepared in GBR solution with concentration ratios of 1, 2, 4, 10, 100, 333 and 1000.

Osmolarities of PEG solutions and peptide solutions (with or without inhibitors) were between 300-309 mosm (**I, II, III**) and 279-311 mosm (**IV**), respectively as determined on an Osmostat® osmometer (Kyoto Kaqaku Co, Ltd, Japan).

4.1.3 Permeability experiments

The rabbits were sacrificed by a marginal vein injection of a lethal dose of T-61 vet. (Hoeschst, Munich, Germany). Initially, the conjunctivas were dissected from the palpebral side (lower eyelid, **I, II, III, IV**) or bulbar side (lower cul-de-sac, **I, II, III**). Thereafter, an incision was made along scleral tissue and corneas were dissected leaving the scleral ring. Lens and iris were removed. The scleras were obtained from an area under the cornea.

The tissues were positioned between two rings and mounted within 25 min **(I, II, III)** or 30 min (**IV**) from sacrifice in the perfusion chamber. The GBR solution was added to the receptor side, and immediately thereafter, an equal volume of the studied compound in GBR was added to the donor side. The pH of the solutions in all permeability studies was adjusted to 7.65 at 37°C by thorough bubbling with O_2-CO_2 (95:5)(**I, II, III, IV**). The sample volumes were 1 ml (**I, II, III**) and 300 µl (**IV**). The samples were collected from the receptor chamber at 30-min intervals. Each sample was immediately replaced with an equal volume of GBR solution. The permeability studies were carried out for 4 hours. At the end of the permeability experiment with the peptide, samples were also taken from the donor side to determine the remaining amount of peptide (**IV**).

The hydration level of the cornea indicated its condition, normally the hydration level was between 76-80 % (Maurice and Riley, 1970). After each corneal permeability study, the hydration levels of corneas were measured in order to check that the corneas had not been damaged. The cornea was removed from the mounting rings, and the remaining sections of scleral and conjunctival tissue were cut away. The cornea was weighed and then dried in an oven at 50°C overnight. After re-weighing the dried cornea, the initial water content was calculated.

4.1.4 The methods for analysing samples

A thermospray (TSP) ionization liquid chromatography-mass spectrometry (LC-MS) method for determination of PEGs in the molecular range from 238 to 986 was developed (**I**). The precision of TSP-LC-MS system was tested using within day precision as described in **I** and day-to-day precision. Day-to-day precision was tested by analysing three same samples (60, 120 and 180 min samples) in six different days. The quantitation limit for PEG 600 was 60 ng per injection. This TSP-LC-MS method was used to determine the concentrations of 17 PEG oligomers in the polydisperse PEG mixtures used in the permeability studies (**III**).

We developed also a gradient HPLC method with combined ultraviolet (UV) and electrochemical detection (ED) for simultaneous analysis of enkephalin peptides, their metabolites, and enzyme inhibitors (Ranta et al., 1997; Ranta et al., 1998). The Waters 486 UV detector (Milford, MA, USA) was set to 205 nm, and was used to assay the enzyme inhibitors, bestatin and SCH 39370. In addition, UV detection was used to determine high concentrations of DAMEA. An ESA Coulochem 5100A electrochemical detector (Bedford, MA, USA) was used with an ESA 5014 flow cell. The first electrode (screen) of the flow cell was set to 0.25 V, and the second electrode (analytical) to 0.60 V. The ED was more sensitive and selective for low concentrations of DAMEA, and its Tyr-containing metabolites than UV-detection. The quantitation limits were; DAMEA, 20 nM; Tyr-D-Ala-Gly, 60 nM; Tyr, 30 nM; bestatin, 500 nM and SCH 39370, 250 nM. The ocular absorption of DAMEA, its metabolites and inhibitors (**IV**) and also the release of D-[Ala^2]-leucine-enkephalin amide and SCH 39370 from PVM/MA-matrices (**V**) were measured by this new method.

4.1.5 Pharmacokinetic parameters (II, III, IV)

The apparent corneal, conjunctival and scleral permeability coefficients (P_{app}, cm/s) were calculated according to equation (1):

$$P_{app} = \frac{dQ}{60\ A\ c\ dt} \qquad (12)$$

Where A is the surface area of membrane (0.28 cm^2 for conjunctivas or 1.17 cm^2 for corneas), dQ/dt is the permeability rate (mg/min **II, III, IV**) of the compound across the studied membrane, c is the initial donor compound concentration (mg/ml **II, III, IV**) and 60 is included to convert minutes to seconds. In addition, P_{app} corresponds to J_h/c, where J_h represents the flux of the compound through the membrane. The lag time (T_{lag}) of permeation was defined as the extrapolated intercept of the linear part of the permeation curve on the time axis (**II, III**).

The expected non-corneal permeabilities of PEGs (through bulbar conjunctiva and sclera) were calculated using equation (**III, IV**):

$$1/P_{nc} = 1/P_{cs} + 1/P_{sc} \quad (13)$$

Where P_{nc} is the permeability in the non-corneal route, P_{cs} is the permeability of bulbar conjunctiva, and P_{sc} is the permeability of sclera.

The corneal and conjunctival clearances for PEGs (µl/min) from lacrimal fluid *in vivo* were estimated using equation (**III**):

$$CL = P \cdot S \quad (14)$$

Where P is the determined corneal or conjunctival permeability (cm/s) and S is the corneal (1.59 cm^2) or conjunctival (15.13 cm^2) surface area (Watsky et al, 1988).

The first-order rate constant for elimination from the tear compartment (K_{el}) was calculated as (**III**):

$$K_{el} = CL/V_d \quad (15)$$

Where V_d is the volume of distribution for PEGs in the tear compartment (i.e. 50 µl).

4.5 Effusion-based theory and estimation of pore sizes and porosity in the membranes (II, III)

Due to the limitations in diffusional modelling and Renkin correction, we developed a new approach for the estimation of the paracellular space. We assumed that the rate-determining step of the paracellular permeability of the drug through the conjunctiva and cornea is the probability for the molecule to find the hydrophilic pores in the tight junctions of the epithelial layer, the rate-limiting barrier for drug penetration. This effusion approach was used to calculate the limiting pore sizes and porosity in the bulbar (**II, III**) and palpebral (**III**) conjunctivas and cornea (**II, III**).

The corneal and conjunctival pore sizes and porosities were calculated using the following equation. The theoretical background and development of the equation have been presented in **II**.

$$\frac{J_h}{C} = \frac{R\,T\,\varepsilon}{12\,\pi\,\eta\,r_s\,N_A\,\lambda} \qquad (16)$$

Where J_h/C is the permeability of PEGs; r_s, the radius of the PEG oligomer molecules; ε, is the porosity of the membrane; λ, jump length of the permeant molecule in water (i.e. 0.31 nm); η, viscosity of water; R, gas law constant (8.3143 J/molK), T, temperature (310 K); and N_A, Avogadro's number ($6.022045 \cdot 10^{23}$/mol).

The measured permeability is inversely proportional to the radius of the compound molecule, and from the slope of this relationship, the porosity of the paracellular space can be evaluated. Extrapolation to a permeability of zero gives the critical value of the pore radius.

The number of pores were calculated from the following relationship:

$$\varepsilon = \frac{A_h}{A} \qquad (17)$$

Where A_h is the effective surface area of the hydrophilic pathways ($A_h = ma_h$; m is the number of paracellular routes in area A, and a_h is the surface area of an individual orifice).

4.3 Release of model compounds from Gelfoam and monoisopropyl ester of PVM/MA matrices *in vitro* (V)

4.3.1 Model compounds

The polymer matrix materials were received as gifts. Gelfoam® sponge was a gift from Upjohn Company (Kalamazoo, MI, USA) and Gantrez ES-335, the monoisopropyl ester of PVM/MA (M_n 21 600) was a gift from GAF Europe (Esher, United Kingdom).

The following compounds were from Sigma Chemical Co (St.Louis, Mo, USA): furosemide (mw 160.1 g/mol), carboxyfluorescein (mw 376.3 g/mol), [D-Ala^2]- leucine enkephalinamide (mw 568.7 g/mol), [D-Ala^2]- methionine enkephalinamide (DAMEA) (mw 586.7 g/mol) and FDs (mw 4400 and 9400). Sodium salicylate (mw 160.10g/mol) was obtained from Aldrich (Steinheim, Germany). Timolol base (mw 316.4 g/mol) was a gift from Interx Research Corp (Lawrence, KS, USA) and timolol maleate (mw 432.5 g/mol) from Merck, Sharp and Dohme Inc. (Rahway, NJ, USA). SCH 39370 (mw 414.4 g/mol) was received as a kind gift from Schering Plough Research institute (Kenilworth, NJ, USA). PEGs (mw 1000 and 4000) were obtained from Fluka AG (Buschs SG, Switzerland) and mannitol (mw 182.2g/mol) from E. Merck (Darmstadt, Germany). The radioactive tracers, which were used with PEGs and mannitol were [1,2-^{3}H]-PEG 931 (specific activity 6.3 mCi/mmol, radiochemical purity 98.4%), D-[1-^{3}H(N)]-mannitol (specific activity 30.0 Ci/mmol, radiochemical purity 99.0%) and [^{14}C]- PEG 4000 (specific activity 60 mCi/mmol, radiochemical purity 99.3%). The two first tracers were from Du Pont, NEN products (Boston, MA, USA) and the last was from Amersham International plc. (Buckingshamshire, UK).

4.3.2 Preparation of matrices

Monoisopropyl ester of PVM/MA matrices were prepared by solvent casting using a 50% isopropanol solution of the polymer (Gantrez® ES-335) as described earlier (Finne

et al, 1989). The polymer solution was mixed in 50 ml of acetone: methanol (1:1). Releasing compound was added and the solution was gently mixed for three hours. When the radioactive tracer was used, the tracer was added at the end of mixing. Thereafter, the mixture was poured onto a Teflon-coated petri dish and allowed to evaporate at room temperature. In the case of buffered matrices, the buffer was dissolved in acetone-methanol mixture with polymer and thereafter, the matrices were prepared in a corresponding manner with the unbuffered matrices.

Gelfoam matrices were prepared in the following way: A small piece of Gelfoam® was cut and placed on petri dish. Water was added so that Gelfoam® had water content of 20%. Gelfoam® was allowed to absorb water and, thereafter, the studied compound was added to the swollen Gelfoam®. Carboxyfluorescein and furosemide were dissolved in methanol and the other compounds were first dissolved in water and then methanol was added. With mannitol and PEGs radioactive tracer was used. The solutions were allowed to evaporate at room temperature.

After evaporation, circular matrices with diameters of 13mm (monoisopropyl ester of PVM/MA matrices) and 18 mm (Gelfoam matrices) were cut with cork borer, kept in a desiccator until used (monoisopropyl ester of PVM/MA matrices) or kept for 24 hours at room temperature in darkness (Gelfoam matrices), and then in a desiccator until used. The dried Gelfoam matrices were 13 mm in diameter.

4.3.3 Drug release and analyses

The rotating disk method was used to study drug release from monoisopropyl ester of PVM/MA matrices and from Gelfoam matrices (Mooney et al, 1981). The speed of rotation was 100 rpm and the temperature was +32°C. The dissolution medium was 200 ml or 100 ml of 2 mM or 40 mM phosphate buffer. The monoisopropyl ester of PVM/MA matrices were attached to the rotating disks with molten block-form paraffin. The Gelfoam matrices were first glued to a piece of Teflon® and then the Teflon piece with the matrix was attached with silicone glue (Dow Corning®, England) to the rotating disks.

The sodium salicylate, timolol, furosemide, carboxyfluorescein and FITC-dextrans were analysed with UV-spectrophotometer (Hitachi 220, Hitachi Ltd, Tokyo, Japan). Mannitol and PEG were analysed by liquid scintillation counting (Rackbeta 1216, LKB Wallac, Turku, Finland). TAGPM, TAGPL and SCH 39730 were analysed with reverse-phase HPLC (Ranta et al, 1997).

Release rates of the drugs (%/min) and the slopes (k) of the log (released drug) versus log (time) plots were calculated from the fitted linear regression lines. In the log-log plot, a slope of 0.5 indicates diffusional square root of time, and a slope of 1.0 indicates zero-order release kinetics (Schwartz et al., 1968). Times of 50% drug release were calculated from the release curves.

4.3.4 Characterization of Gelfoam

The buffer solution uptake (%) in Gelfoam matrices was studied using the rotating disk method in the same conditions as used in the drug release tests. The matrices were weighed dry and during hydration at the same intervals as in the release studies. The buffer uptake was expressed as the weight of buffer absorbed by a unit weight of the wet matrix multiplied by 100%.

When swelling was studied the initially dry samples were allowed to swell in milli-Q water, in 2 mM or 40 mM phosphate buffer (100 ml). The pH was 7.4 and temperature +32°C. the discs were removed after 10 minutes and then at 30 minute intervals. The thickness and diameter of discs were measured manually and the discs were returned to the solutions to continue swelling. The effective diffusional area (S) and the effective volume of the swollen matrix (V) were calculated and the exposed surface area to volume ratio (S/V) of Gelfoam matrices was determined at different time points.

The charge of Gelfoam at different pH-values and buffers was determined by calculating the transport number of potassium (t^{K+}). The Gelfoam matrices were allowed to swell for 1-1.5 h in water or in 2 mM or 40 mM phosphate buffer at pH 3.0, 6.0, 7.4. or 10.0. The matrices (Ø 18 mm) were positioned between donor and receptor compartments of side-by-side glass diffusion cells (Crown Glass Company, Inc., Somerville, NJ). In the donor side was 0.01 M KCl-solution (3.0ml) and in the receptor side there was 0.1 M KCl-solution (3.0 ml). The osmotic pressure was regulated by

sucrose to 171-179 mOsm/kg and the temperature was +32°C. Potential differences across the Gelfoam matrix were measured with a microvolt multimeter (Hewlett Packard F 2378 A, Japan) and Ag/AgCl-electrodes. The measurements were continued until the potential started to decrease. The transport number of potassium (t^{K+}) was calculated using the following equation (Hirvonen et al., 1993):

$$E = \frac{2\, t^{K+} R\, T}{Z_+ F} \ln (a^I/a^{II}) \qquad (18)$$

Where E is electrical potential, a^I and a^{II} are activities of the salt in two solutions, and t^{K+} is the transport number of potassium in the membrane, T is the temperature, Z_+ is the charge and F is the constant of Faraday. The t^{K+} is 0.50 in water and in neutral membrane and t^{K+} is higher than 0.50 in negatively charged polymer and less than 0.50 in a positively charged polymer (Rojanasakul and Robinson, 1989).

4.4. Statistical analyses

The statistical significance between the permeabilities of different PEG oligomers through the membrane was tested using one-way analysis of variance (**III**). Values of $p < 0.05$ were considered to represent statistically significant differences.

Mann-Whitney's U-test was used to test statistical significance of the differences between drug release *in vitro* from the matrices with and without sodium acetate (**V**). The effects of the pH of the buffer on drug release were tested with one way analysis of variance (ANOVA)(**V**).

5 RESULTS AND DISCUSSION

5.1 Characterization of paracellular route in ocular membranes

5.1.1 Analysis of PEG-oligomers with TSP-LC-MS-method

The suitability of the quantitation range of PEGs for the *in vitro* permeation studies was tested with two conjunctival permeability studies (**I**). The precision of the TSP-LC-MS system was tested using day-to-day precision by analysing three same samples (60 , 120, and 180 min samples) on six different days. The relative standard deviation (RSD) was biggest in the 60 min PEG samples (RSD range 12.8 - 29.8%) since the concentrations in the receptor side were small. At 120 and 180 min the RSD range was 4.7- 25.6%. The precision of the TSP-LC-MS method was best for PEGs in the mw range from 238 to 810 (RSD from 4.7% to 19.8%). The larger the mw, the poorer was the precision. Thus, the TSP-LC-MS method allows determination of P_{app} for a range of PEGs in the mw range 238-942. The quantitation limit of the method for PEG 600 was 60 ng per injection.

5.1.2 Permeability of anterior membranes

Cornea. Corneal permeability of PEGs decreased with increasing molecular weight (**III**, Fig 2A). The P_{app} was almost five fold higher with PEG of mw 238 (P_{app} 1.03 x 10^{-6} cm/s) than with PEG of mw 942 (P_{app} 0.22 x 10^{-6} cm/s) (**III**, Fig 2A). The permeability decreased most significantly at molecular weights below 414. At higher molecular weights, the permeability decreased to a lesser extent.

Liaw and Robinson (1992) observed the permeability remained essentially constant (P_{app} range 1.13-1.04 x 10^{-6} cm/s) for PEGs between molecular weights 206-415. Between mw 400-600 the corneal permeability decreased rapidly with increasing molecular weight but above 600 P_{app} decreased only slowly. Permeability results of Liaw and Robinson (1992) differ from this study for several reasons. Liaw and Robinson (1992) used approx. 10 times greater initial PEG concentrations in the donor side, their sample bathing medium was 0.16 M NaCl and the analytical method was

HPLC with refractometric detection. In that study the standard errors of corneal P_{app}s in the mw ranges of 200-300 and 850-950 (Liaw and Robinson, 1992) were much greater than in our study. This may be due to the fact that the refractometric HPLC method is hundred times less sensitive than our TSP-LC-MS method. Furthermore, in concentrated solution, PEG molecules associate, and the chains of low molecular weight PEG are less ordered than bigger ones (Liu and Parsons, 1969). The probability for self-association of polymers increases with their molecular weight, concentration and ionic strength. This may explain the abrupt fall in permeability values at mw 400-600 observed by Liaw and Robinson (1992). In dilute solutions, PEG chains are expanded (Graessley, 1980) and it has been suggested that the PEG polymers fold when the molecule is longer than 12 -14 units (mw range approx. 546-634) (Hollander et al., 1988).

The same kinds of observations as in noted our study have been made also in the PEG permeability studies in gastrointestinal walls of humans and rats (Chadwick et al., 1977; Hollander et al., 1989; Donovan et al., 1990). These workers reported a progressive decrease in urinary recovery of PEGs between molecular weights 200 and 400. This rather constant permeation such as we had above mw 500 of PEGs (and also Liaw and Robinson, 1992) has been suggested to be due to the water solubility (Ma et al., 1990). Hollander et al. (1989) and Ma et al. (1990) observed a linear decrease in the PC with increasing mw of the fractions between 200 and 1000 (logPC –4.00- -2.00) indicating increasing relative water solubility of individual mw fractions between 200-1000.

Conjunctivas. The palpebral and bulbar permeabilities of PEGs were equal at each mw. In general, the conjunctivas were approx. 15-25 times permeable to PEGs than the cornea (**III**, Figs 2A, B, C) and increasing mw decreased the permeation of PEG, but the effect of mw was minor (appr. 2-3 fold). In palpebral conjunctiva, the P_{app} of PEG 238 was 1.65×10^{-5} cm/s and P_{app} of PEG 942 was 0.70×10^{-5} cm/s. The corresponding P_{app} of PEG 238 and 942 in the bulbar conjunctiva was 2.16×10^{-5} cm/s and 0.60×10^{-5} cm/s, respectively. The effect of the mw of the molecule was less apparent in conjunctival permeability than in corneal permeability.

The results are in accordance with other reports. The conjunctival permeabilities of hydrophilic compounds are usually 10-100 times higher than those in the cornea (Ahmed et al., 1987; Huang et al., 1989; Sasaki et al., 1995c; 1997). It is not surprising that the rates of permeation in palpebral and bulbar conjunctivas were equal for PEGs (mw < 1000). All three conjunctival epithelia have essentially the same structure and they are maintained by the same population of stem cells (Wei et al., 1993). Horibe et al. (1997) studied the conjunctival permeation of hydrophilic compounds (mw varying between 4400–167 000). They noted an inverse relation between P_{app} (range 27.7 x 10^{-8}-0.3 x 10^{-8}cm/s) vs. molecular size (radii of the compounds 0.43-4.94 nm). They suggested that hydrophilic compounds in the mw range of 4400 to 40 000 probably traversed the conjunctiva by restricted passive diffusion and that the endocytotic pathway may be responsible for permeation of dextrans with mw > 70 000.

Sclera. The scleral permeability of PEGs was approx. half of that in the conjunctiva, and approx. 10 times greater than in the cornea (**III**, Figs. A, B, C, D). The PEG permeability decreased approx. 3 times between mw 238 (P_{app} 8.80 x 10^{-6} cm/s) and 942 (P_{app} 3.08 x 10^{-6} cm/s)(**III** Fig. 2D). The sclera has been found also earlier to be more permeable than cornea for hydrophilic compounds (Ahmed et al., 1987; Sasaki et al., 1995a, b, c).

The higher scleral permeability, as compared to the cornea, is due to the fact that the PEGs in our study were small enough to permeate through the sclera. Only tortuosity of the water channels affected the PEG permeability. Rather surprisingly it has been claimed that the solute size has a more pronounced effect on the scleral permeability (Ahmed et al., 1987) than corneal permeability. According to Prausnitz and Noonan (1998) the permeability is size restricted only at molecular radii over 1.0 nm. In our study, the mw had clearer effects on permeability in cornea than in sclera. This is understandable, since the cornea has tight junctions, while there are not so tight anatomical barriers in the sclera.

Non-corneal route. The calculated permeability of the non-corneal route (bulbar conjunctiva + sclera) was calculated to be 6.25 x 10^{-6} cm/s for PEG 238 and 2.03 x 10^{-6} cm/s for PEG 942. Both PEGs would permeate better through the non-corneal route than through the cornea. The P_{app}s of PEG 238 and PEG 942 are six and nine times

respectively higher in the non-corneal compared to the corneal route. This supports the idea that conjunctiva/sclera is a more important pathway than the cornea for the absorption of hydrophilic and large molecules into the eye.

5.1.3 Estimation of pore size and porosity of the epithelia

The diffusion approach and the use of Renkin correction resulted in values of 0.7-0.8 for the r_d/r_p ratio (radius of diffusing molecule/efficient pore size). The pore size calculations were based on the results of the *in vitro* permeability studies with PEGs and mannitol was used as the reference compound. When Renkin correction is valid, r_d/r_p should be not greater than 0.3-0.4 (Davidson and Deen, 1988). Therefore, the diffusional approach rather than the Renkin correction should not be used in this case.

If one assumes that the densities of the pores in the conjunctiva and cornea are low (Nichols et al., 1983) then permeating hydrophilic molecules will most probably collide with the cell membrane and only occasionally find a paracellular pore to permit permeation. Therefore, we developed an effusion-based theory assuming that the low probability of finding the pore, rather than diffusion, determines the paracellular permeation. This effusion approach can be used to calculate the pore sizes in the biomembranes if they have a very small effective barrier thickness (e.g. 1-2 cell layers) and, secondly, the fraction of the intercellular spaces in the total epithelial area is very small. Cornea and conjunctiva fulfil these criteria, but sclera does not.

In the sclera, the primary mechanism for drug absorption is likely to be diffusion across the fairly long aqueous tortuous meshlike structure. Tortuosity and the space in the water channels would be predicted to determine the drug permeability.

In effusion-based calculations (**II**, Eqn. 9) the radii of permeating molecules were used. The radii of PEG were calculated on the basis of the radius of gyration ($r_s = 0.7\ r_g$), which takes into account the solvent properties. The r_s^* radius of PEG was calculated with Stokes-Einstein equation (**II**, Eqn. 10). Applying the effusion approach to the permeation data of PEGs, a pore size of 1.0/0.73nm (r_s/r_s^*), a porosity of 1.3/2.1 $x10^{-7}$, and a pore density of 4.26/12.5 x $10^6/cm^2$ were obtained in cornea (**II**, Table 2, Fig 2a). The corresponding values in bulbar conjunctiva were pore size 1.53/0.88 nm, porosity 2.45/3.75 x 10^6, and pore density of 33.3/154 x 10^6 /cm^2 (**II** Table 2, Fig 2b, **III**). In

palpebral conjunctiva, the pore size was estimated to be 2.45 nm on the basis of r_s (**III**). We calculated also the paracellular surface areas in the epithelia. In the cornea (area 1.59 cm^2), the total area of the paracellular route was 0.03 x 10^{-3} mm^2 and in the conjunctiva (area 15.1 cm^2) it was 0.007 mm^2 (**III**).

The epithelial pore sizes in the eye are in the same range as the estimates in the other epithelia (pore range 0.39 –1.7 nm) (Taylor and Gaar, 1970). Our calculation for the critical pore diameter in cornea (2.0 nm/1.5 nm) (**II** Table 2, Fig 2a, **III**) is in line with earlier observations. The earlier estimations for the paracellular pore size in the cornea have varied between 1.2 nm to 3.0 nm (Grass and Robinson, 1988a and b; Lee et al., 1986b; Tonjum, 1974).

Horibe et al. (1997) published their estimations of pore size and pore density in pigmented rabbit conjunctival epithelium. They suggested that polar solutes up to 40 000 traverse the conjunctival epithelium primarily by restricted diffusion through equivalent pores of 5.5 nm in radius. The pore density in the conjunctival epithelium was proposed to be 1.9 x 10^8 pores per cm^2. The P_{app}s decreased with solute size, from 27.7 x 10^{-8} cm/s for mannitol (mw 182, diameter 0.43 nm) to 0.31 x 10^{-8} cm/s for FD150 (mw 167 000, diameter 4.94 nm). The permeability values are much lower than other published values (range 10^{-6} cm/s) (Sasaki et al., 1995a, b, c; Prausnitz and Noonan, 1998). Although Horibe et al. (1997) noticed very high TER in conjunctiva and the hydrophilic compounds permeated very poorly, the pore size in the conjunctiva was found to be 11.0 nm (in this study 1.8-3.1 nm)

5.1.4 Corneal and conjunctival clearance

In addition to the solution drainage, also drug absorption clears drug from the lacrimal fluid. The contribution of the paracellular absorption to the clearance from lacrimal fluid was estimated. Clearances were calculated on the basis of the permeabilities and surface areas of the membranes as described earlier (Urtti et al., 1990). The paracellular corneal clearances (µl/min) from the tear compartment were estimated to be 0.1 µl/min (K_{el} = 0.20%/min) for PEG 238, and 0.02µl/min (K_{el} = 0.04%/min) for PEG 942 (**III**). The corresponding palpebral conjunctival clearances

from the lacrimal fluid were; clearance of PEG 238, 15.0 µl/min (K_{el} = 30%/min) and clearance of PEG 942, 6.3 µl/min (K_{el} = 12.7%/min) (**III**). The paracellular elimination from the tear fluid through conjunctiva is much greater than the elimination through the cornea.

In comparison, preocular clearance values of pilocarpine, timolol, and flubiprofen are more than 10µl/min (Urtti, 1995). Clearance values were calculated using the total surface areas of the conjunctiva and cornea. *In vivo* the applied compound is not in contact with the entire conjunctiva. Therefore, the above values may overestimate the clearance from lacrimal fluid via paracellular absorption. However, the clearance via paracellular absorption is substantial and contributes to drug removal from the lacrimal fluid.

5.2 Permeation of peptides in ocular membranes (IV)

5.2.1 Analysis of peptides

A gradient HPLC method with combined UV and electrochemical detection (Ranta et al., 1997) was developed to determine the ocular permeability of DAMEA *in vitro*. The method allowed the determination of DAMEA, several of its metabolites as well as the enzyme inhibitors bestatin and SCH 39370 in the same run. ED was more sensitive and selective than UV detection at 205 nm for DAMEA and its Tyr-containing metabolites. The detection limits (S/N 3) of ED for MEA and its Tyr-containg metabolites were 8-12 nM (0.4-0.6 pmol/50 µl injection). The detection limit of DAMEA in UV detection (1.0 pmol) was two times higher than in ED. The linear dynamic range of UV detection extended to higher concentrations, which was useful when analysing conjunctival permeability samples with high levels of DAMEA. UV detection was also used to determine the non-electroactive enzyme inhibitors bestatin and SCH 39370. A typical chromatogram is shown in Fig 6. The gradient HPLC with combined UV and electrochemical detection was a sensitive and selective method for permeability and metabolic studies of DAMEA in cornea, conjunctiva and sclera *in vitro*.

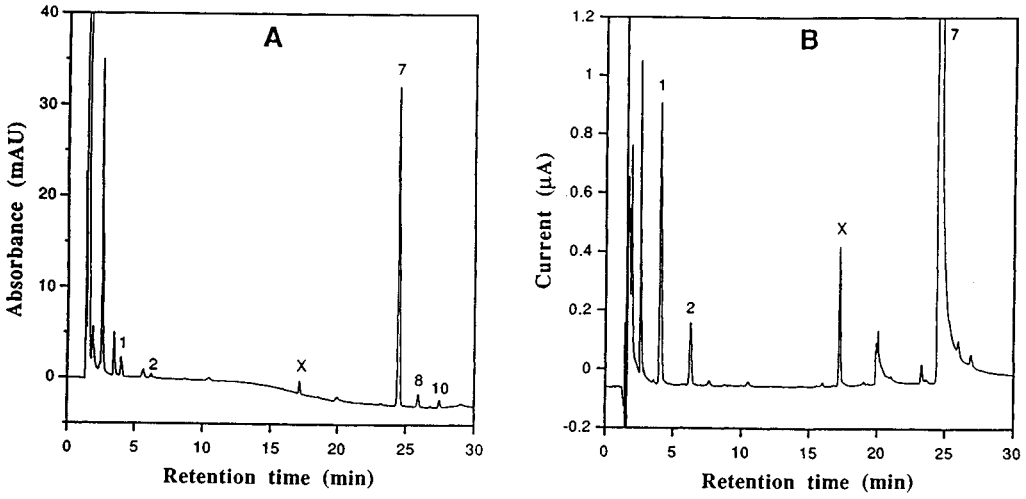

Figure 6. Analysis of DAMEA, its metabolites, and enzyme inhibitors in the receptor side at the end (4h) of in vitro conjunctival permeability experiment with inhibitors. A. UV detection at 205 nm, B. ED at 0.60 V. peaks: 1. Tyr (0.599 µM); 2. Tyr-D-Ala-Gly (0.177 µM); 7. DAMEA (10.9 µM); 8. SCH 39370 (3.54 µM); 10. Bestatin (4.03 µM); X. matrix component. Initial donor side concentrations: DAMEA 1.0 mM, bestatin 0.25 mM, SCH 39370 0.25 mM.

5.2.2 Cornea

Cornea was almost impermeable to DAMEA without inhibitors. Only 0.007 ± 0.002% of DAMEA permeated to the receptor side (**IV**, Fig 1a.). When the inhibitors, bestatin and SCH 39370 were coadministered with DAMEA to the donor side, the permeation of DAMEA increased 15 times (0.07% ± 0.02%), P_{app} was 0.40 x 10^{-6} cm/s (**IV**, Table 1, Fig 1a).

The major metabolites of DAMEA were Tyr and Tyr-D-Ala-Gly (**IV**, Fig 2a). Concentrations of Tyr and Tyr-D-Ala-Gly exceeded those of DAMEA (**IV**, Fig. 2a). This may be due to extracellular and membrane bound peptidases (Turner and Tanzava, 1997; Grady et al., 1997; Piva et al., 1998; Roques 1998). The inhibitors blocked almost completely the formation of those metabolites (**IV**, Fig 2a). Some of the Tyr was released from the control cornea. The permeabilities of bestatin and SCH 39370 were higher than that of DAMEA with inhibitors. It is important that the activities of both aminopeptidase and endopeptidase are inhibited. When only the aminopeptidase

inhibitor was administered with enkephalin, no increase in absorption was found (Lee et al., 1986a).

The corneal permeability of DAMEA with the inhibitors was in the same range (or slightly smaller) than the corneal permeability of PEG with similar molecular dimensions (**III**, Fig 2A). This suggests that DAMEA permeates through cornea via paracellular pathway and that the low pore density limits the permeability. Since DAMEA is hydrophilic (log PC 0.34), it cannot permeate through the cells (Bohner Lang et al., 1997). Therefore, size limited paracellular permeability is the theoretical maximum for its permeability without metabolism. The molecular size of DAMEA (≈ 0.5 nm in radius) is clearly smaller than the limiting size of the paracellular pores in the cornea.

5.2.3 Conjunctiva

DAMEA permeated through rabbit conjunctiva 114 times faster than through the cornea (**IV**, Table 1). When the peptidase inhibitors were present, the conjunctiva was 42 times more permeable than the cornea with inhibitors. The concentration of DAMEA in the receptor side at 4h was 6.3 ± 0.8 μM (0.63 ± 0.08%). Tyr and Tyr-D-Ala-Gly were the major metabolites in the conjunctiva and some of the Tyr was released from the control conjunctiva (**IV**, Fig. 2b). The concentrations of Tyr and Tyr-D-Ala-Gly exceeded those of DAMEA as in cornea (**IV**, Fig. 2b). With peptidase inhibitors, the conjunctival permeability of DAMEA increased 6 fold (from 3.1 x 10^{-6} cm/s to 16.9 x 10^{-6} cm/s)(**IV**, Table 1). In the conjunctiva, the inhibitors blocked almost completely the formation of metabolites (**IV**, Fig. 2b). Bestatin permeated 1.5 times better and SCH 39370 permeated 1.4 times better in the conjunctiva than DAMEA with inhibitors (**IV**, Table 1).

The molecular size of DAMEA should not limit its permeation through the conjunctiva to the same extent as in the cornea, because the paracellular pores are bigger in the conjunctiva and the pore density is also greater. As in the cornea, the permeability of DAMEA with peptidase inhibitors was similar to the permeability of PEG with

similar molecular dimensions emphasizing the critical role of paracellular pore size and pore density (**III**, Fig. 2C).

5.2.4 Sclera

Sclera was 463 times more permeable to DAMEA without inhibitors than cornea and four times more permeable than the conjunctiva (**IV**, Table 1). When there was no inhibitors, the concentration of DAMEA was 26.7 ± 12.3 μM (2.7 ± 1.3%) after 4 h permeability study. With enzyme inhibitors present, the permeability of DAMEA did not change (**IV**, Table 1). The primary metabolites in the sclera were Tyr and Tyr-D-Ala-Gly, but the permeation of metabolites in sclera was smaller than in the cornea and conjunctiva (**IV**, Fig 2a, 2b, 2c). Some Tyr was also released from the control sclera. Although the enzyme inhibitors did not affect DAMEA permeation, the formation of the primary metabolites was reduced (**IV**, Fig 2c). It seems that the sclera has lower enzymatic activity than the cornea and conjunctiva. Bestatin permeated through sclera 1.7 times and SC 39370 permeated 1.5 times better than the DAMEA with inhibitors (**IV**, Table 1).

Non-corneal route. The estimated permeability of the non-corneal route (conjunctiva + sclera) was calculated to be 2.48 x 10^{-6} cm/s for DAMEA without inhibitors and 6.36 x 10^{-6} cm/s for DAMEA with inhibitors. The estimated non-corneal permeability of DAMEA was without 92 times and with peptidase inhibitors 16 times higher than in the cornea (**IV**, Table 1). Conjunctiva/sclera is a more viable route for the pentapeptide delivery into the eye than the cornea. Combination of aminopeptidase inhibitor and neutral endopeptidase inhibitor appears to be an efficient means to improve peptide permeation in ocular membranes.

5.3 Drug release from ocular inserts

5.3.1 Characteristics of monoisopropyl ester of PVM/MA matrices

Effect of mw. The mw of the releasing compound did not affect drug release from monoisopropyl ester of PVM/MA matrices (**V**, Fig. 1). The release rate of PEG 1000

and PEG 4000 from monoisopropyl ester PVM/MA matrices was 1.45 %/min and 1.50 %/min in 40 mM phosphate buffer, respectively. The release of PEGs conformed to zero order kinetics (k=0.9-1.1). When the PEG content was decreased from 100 mg to 10 mg, the release rate increased 1.4 fold and $t_{50\%}$ decreased from 45 min to 34 min ($p < 0.05$). The results suggest that PEG release from the matrices is controlled by polymer relaxation or surface erosion of polymer. The drug is leached from monisopropyl ester of PVM/MA matrices from the undissolved matrix portion at a constant rate possibly due to solvent penetration at the same time as the matrices are undergoing surface erosion (**V**, Fig. 1, Table 3).

Monoisopropyl ester of PVM/MA matrices dissolved completely in 40 mM phosphate buffer during the release test and in general the drug release from matrices was faster in 40 mm phosphate buffer than in 2mM (**V**, Table 2, Fig. 3 and 4). The difference was several fold and it was due to the effects of buffer on polymer surface pH (Finne et al., 1989; Heller et al., 1978).

Effect of acid/base nature. It was found that the acid/base nature of the drug affects the monoisopropyl ester of PVM/MA dissolution and drug release from the matrix (**V**, Table 3, Fig. 3). Sodium salicylate (pKa ≈3) was released significantly faster than furosemide (pKa 3.9) ($p < 0.05$) and timolol maleate (pKa 9.2) in 2 mM phosphate buffer (**V**, Table 3, Fig. 3). It appears that the salt of an acid (sodium salicylate) is released faster than that of a weak acid (furosemide) or salt of a weak base (timolol maleate). Timolol base was released faster than timolol maleate (**V**, Table 3, Fig. 3). These differences are probably due to the effects of microclimate pH on the polymer surface. The bases raise the surface pH and accelerate polymer dissolution, and thus facilitate their own release.

Effect of matrix buffering. The release rates of sodium salicylate, timolol base, timolol maleate and furosemide increased by approx. 2 fold with addition of sodium acetate to the matrix (2 mM buffer, pH 7.4)(**V**, Table 3, Fig. 3). Sodium acetate did not significantly affect the bulk pH of dissolution medium during the test. The release of the compounds was by erosion of the polymer matrix (zero-order)(**V**, Table 3). Buffering of the monoisopropyl ester of PVM/MA matrix increased the release rate as suggested previously (Finne et al., 1989; Finne et al., 1992).

Peptides and monoisopropyl ester of PVM/MA matrices. DAMEA degraded during the solvent casting process and this was found as a double peak in HPLC-analysis. The degradation product was identified to be methionine-enkephalin sulphoxide by using HPLC ESI-MS/MS. It appears that methionine-containing peptides are incompatible with the monoisopopyl ester of PVM/MA polymer. The degradation problem was solved by using [D-Ala^2]–leucine-enkephalinamide. The release of [D-Ala^2]–leucine-enkephalinamide and enkephalinase inhibitor SCH 39370 from monoisopropyl ester of PVM/MA matrices was zero-order (k =0.9-1.0)(**V**, Table 4, Fig. 5). When SCH 39370 was combined with [D-Ala^2]–leucine-enkephalinamide, both of them were released at the same rate (**V**, Table 4, Fig. 5).

5.3.2 Characteristics of Gelfoam matrices

Effect of mw. The mw of the compound clearly affected its release rate from Gelfoam matrices (**V**, Table 2). The drug release mechanism was Fickian diffusion or swelling controlled (non-Fickian) transport (k=0.3-0.6)(**V**, Table 2). In general, the higher the mw of the compound, the slower was the release (**V**, Table 2, Fig. 2). One exception was carboxyfluorescein, which was released from the matrices at the same rate as FITC-dextrans, Half of carboxyfluorescein and FITC-dextran were released from Gelfoam matrices about in 60 min (**V**, Table 2). The reason for this may be the electrostatic attraction between the carboxylic groups of the carboxyfluorescein and the positive charge in gelatin.

The Gelfoam matrices did not dissolve in 2 mM or 40 mM buffer during the tests, but they did swell. The diffusion length for the studied compounds was shorter in 40 mM phosphate buffer (the matrices swelled less in 40 mM buffer) than in 2 mM, and this could be one reason that in general the compounds were released more rapidly from the 40 mM buffer. The exceptions were carboxyfluorescein and FITC-dextrans. Their release rates were equal in 2 mM and 40 mM buffer. Generally about 50% of the studied compounds were released in an hour, and total release of compounds continued for four hours.

Effect of acid/base nature and pH of buffer. The drug release from Gelfoam matrices was affected by the pH of buffer (**V**, Table 2). Depending on pH, acids or bases were released faster. At pH 7.4 furosemide was released much faster than timolol (**V**, Table 2). At pH 6.0 timolol was released much faster than furosemide. The reason for this could be that furosemide and timolol may change the Gelfoam charge and, thereby, modify their own release from the matrix. The release rate of negatively charged carboxyfluorescein and FITC-dextrans was not affected by pH. The lack of pH effects may be due to the greater role of hydrophobic interactions between these compounds and Gelfoam.

Hydration and swelling. Gelfoam matrices became soft within a couple of minutes when the buffer permeated into the matrices (**V**, Fig 6 and 7). They swelled in 10 min and slow swelling continued for 90 min, when the buffer content of the matrices became equilibrated. During swelling, the radius of the matrices did not change, only the thickness increased (**V**, Fig. 7). The S/V ratio increased rapidly to the maximum and then dropped to a lower value and remained fairly constant after 90 min (**V**, Fig. 7). Swelling was less in 40 mM phosphate buffer. The presence of buffer produces more osmotically active species in the solution than in water. The osmotic pressure difference between gelatin and buffer becomes reduced, which results in less swelling in the buffer solution than in water. The drug release followed the swelling process (**V**, Fig 2). During the first 10 min, when the swelling process was at its fastest, all compounds were released equally and thereafter, the release was affected by the mw. The latter phase was probably controlled by diffusion in the hydrated polymer.

Charge. On the basis of the transport number of potassium ions, Gelfoam was usually uncharged in the test buffers (t^{K+} = 0.50 which is similar to t^{k+} in water) (**V**, Table 5). The only exception was that in 40 mM buffer at pH 6.0 Gelfoam was positively charged i.e. slightly anion selective (t^{K+} = 0.44)(**V**, Table 5). The results suggest that the effective charge in Gelfoam may change from neutral to positive depending on test conditions. This affects the release of drugs from Gelfoam matrices as shown with furosemide and timolol.

5.3.3 Monoisopropyl ester of PVM/MA and Gelfoam matrices as potential ocular drug delivery systems

An ideal ocular drug delivery system should possess the following practical characteristics; ease of handling and application, safety, tolerability, lack of expulsion during use, appropriate release kinetics and applicability for a variety of drugs. In addition, the system should be easy to manufacture, stable and cheap. In this study, the release kinetics of two polymeric materials was compared.

The advantages of monoisopropyl ester of PVM/MA matrices are the constant drug release, the possibility of control release rate of drug by buffering or derivatisation. The matrices show prolonged action, and bioerodible matrices do not have to be removed after use. Interestingly, the polymer governs drug release from these matrices, not the releasing drug. *In vivo*, it has been proposed that the surface of monoisopropyl ester of PVM/MA matrices softens within a few minutes after application in the eye (Finne et al., 1990). Furthermore, the inserts did not cause any irritation when placed in the lower conjunctival sac of rabbit eye. *In vivo,* however, erodible systems can have significantly variable erosion rates based on individual patient physiology, lacrimation patterns and buffering capacity of the tear fluid (Bawa and Nandu, 1990).

The Gelfoam matrices softened very rapidly when they came into contact with water. The Gelfoam devices have been well tolerated by rabbits: blinking was observed for only a few seconds (Pinsuwan et al., 1997; Lee et al., 1997) before the device became hydrated. In comparison, Gelfoam devices with embedded cetyl ester wax induced blinking for 1 min (Simamora et al., 1998). No physical signs of irritation (e.g. redness, lacrimation) were observed during any *in vivo* experiment. The manufacture of Gelfoam matrices is simple and application into the eye would be easier than with monoisopropyl ester of PVM/MA matrices due to their softness. In our study the mw (ionization, charge) affected the rate of drug release from the Gelfoam matrices, but the drugs were released for a few hours regardless of mw of drugs. Gelfoam matrix prolongs the contact time of the drug with the eye.

The disadvantages of Gelfoam matrices include non-constant drug release by diffusion. The removal of matrix might be necessary after drug release, although it has been reported that Gelfoam devices have been not found in the cul-de-sac after the

second day of instillation (Lee et al., 1997a). The long-term effects of Gelfoam on the ocular tissues are currently not known.

Large molecules such as oligonucleotides, peptides and proteins pose the great challenges in ocular drug delivery. It appears that the conjunctiva and sclera (non-corneal route) is a more viable route than the corneal route for delivery of large molecules. Conjunctiva and sclera are more permeable and they are enzymatically less active than the cornea. The polymer matrices are placed in the conjunctival sac, which prolongs their contact time with the conjunctiva and, therefore, they may improve the delivery of large molecules via the non-corneal route into the eye.

6 SUMMARY AND FUTURE ASPECTS

Although monoisopropyl ester of PVM/MA and Gelfoam matrices seem promising *in vitro*, e.g. in terms of drug release, the situation can be different *in vivo*. The matrices certainly prolong the contact time on the ocular surface, but there are some open questions, can be solved only in further long-term studies. These include the ocular bioavailability, systemic drug absorption, long-term drug and excipient stability in the formulations, and tolerability of the matrices during multiple dosing to the eyes.

In the past, administration of ocular drugs in polymeric inserts has resulted in moderate success. The first clinically used controlled release system in ophthalmology was the Ocusert®, a reservoir that delivered pilocarpine for one week and controlled intraocular pressure with less drug and fewer side effects compared to the eyedrop treatment. Despite its advantages, Ocusert® never achieved widespread use, due to its expensive price and poor acceptance by older patients (Langer R, 1990). Nowadays, there is only one clinically used ocular matrix system in Finland. Lacrisert® is used to treat the dry eye syndrome.

The preparation of monoisopropyl ester of PVM/MA and Gelfoam matrices was easy in laboratory scale but industrially it is more demanding. Production of new dosage forms require new production lines in the pharmaceutical industry, standard production units can not be utilized.

The non-corneal route is more permeable than the cornea for hydrophilic compounds *in vitro* in rabbits. These conclusions may not, however, be directly applicable to *in vivo* situation in humans. For example, the blood circulation in the anterior segment may drain part of the non-corneally permeating drug. Nevertheless, administration of hydrophilic drugs, like peptides and oligonucleotides, via non-corneal route using locally applied polymeric systems is an interesting possibility in the treatment of ocular diseases.

7 CONCLUSIONS

This study shows that the non-corneal route is physically permeable and enzymatically more preferable than the corneal route for absorption of hydrophilic molecules into the eye. The major conclusions of this study are as follows:

1. The TSP-LC-MS method is suitable for the analysis of permeation of PEGs through biomembranes in the molecular weight range of 238-942 g/mol. The method is useful for studies of the molecular weight dependence in paracellular membrane permeation.

2. An effusion-based approach was useful in the determination of paracellular pore diameter, porosity and the number of pores in the biomembranes. Paracellular chracteristics of cornea and conjunctiva were solved using effusion-based approach.

3. The conjunctival epithelium has larger paracellular pores and it is more porous than the corneal epithelium.

4. The paracellular permeability in the non-corneal conjunctival/scleral route was significantly higher for PEGs and pentapeptide than the corneal permeability. Combination with inhibitors of aminopeptidase and neutral endopeptidase increased the permeation of DAMEA by 1-2 orders of magnitude substantially in the cornea and conjunctiva. The cornea was enzymatically most active tissue, sclera had the lowest peptidase activity. The non-corneal route is a more viable pathway for ocular peptide and oligonucleotide delivery than the cornea. This advantage could be utilized with a dosage form that targets the absorption to these membranes.

5. Monoisopropyl ester of PVM/MA matrices released drugs at a constant, zero-order rate. The acid/base nature of the drug affects the polymer dissolution and drug release rate from monoisopropyl ester of PVM/MA matrices. Sodium acetate increases the release rates of drugs without changing the release profile (zero-order). The molecular weight of the drug did not affect the release from monoisopropyl ester of PVM/MA matrices, and therefore they may be suitable vehicles for controlled delivery of peptides and oligonucleotides into the eye.

6. Diffusion or polymer swelling controlled drug release from Gelfoam matrices. The release obeyed a square root of time relationship. The properties of the drug (molecular weight, charge, ionization etc.) also affected drug release. Gelfoam® is inert, easy to handle and the matrices are simple to manufacture. Disadvantages include its insolubility and non-constant release rate that also varies with the molecular weight of the drug.

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