CHAPTER 2
LITERATURE REVIEW

2.1 Anatomy and function of the eye

Anatomy of the eye is shown in Figure 2.1.The cornea is the clear surface of the outer eye. It is transparent, avascular tissue to which nutrients and oxygen are supplied by the lachrymal fluid and aqueous humour [6]. It is about 0.5 mm thick and consists of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane and the endothelium layer. It has two main functions. Firstly, it acts as a barrier preventing germs, dirt and other harmful material from entering the inner eye. Secondly, the cornea acts as the eye's outermost lens. It functions like a window that controls and focuses the entry of light into the eye. The cornea contributes between 65-75 percent of the eye's total focusing power and when light strikes the cornea, it bends, or refracts, the incoming light onto the lens. The cornea is considered to be the main pathway for the permeation of drugs into the eye. Drugs penetrate across the corneal the corneal epithelium via the transcellular or paracellular pathway [34].

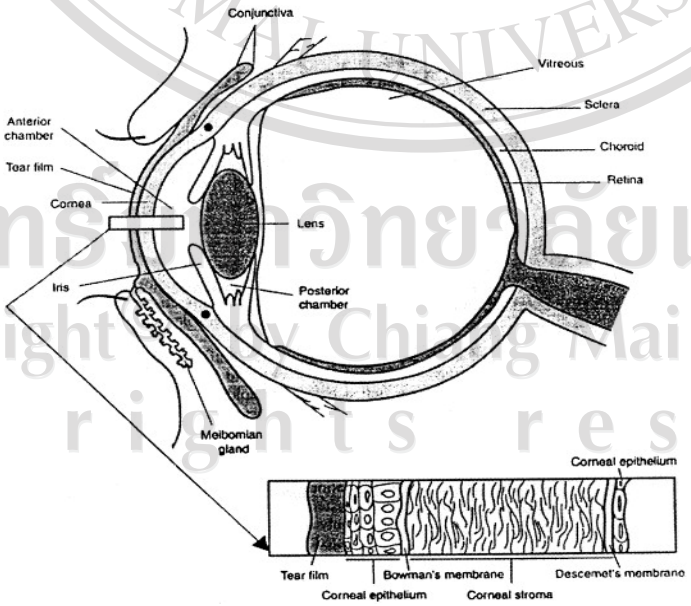

Figure 2.1 Anatomy of the eye [6].

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The iris gives the eye its colour. This colour is genetically determined. It is made up of three layers of connective tissue and muscle fibers: endothelium, stroma and the epithelium. Its main function is to control the amount of light that is let into the eye. In bright light, the muscles contract, which causes the opening at the centre of the iris (the pupil) to constrict. In dim light the muscles dilate, thus allowing more light into the eye.

The pupil is the opening at the centre of the iris that lets light into the eye. It changes size in response to light levels.

The lens is a transparent structure of about 5 mm thick. It has a diameter of approximately, 9 mm is positioned directly behind the iris, and is made of proteins called crystallins. Its function is to focus light onto the retina. The lens is flexible and its curvature is controlled by the nervous system through surrounding muscles. The changing curvature of the lens allows the eye to focus on objects at different distances. The lens is encased in a capsule and suspended within the eye by zonule fibres.

The vitreous humour is a clear, thick, substance that fills the centre of the eye. It is comprised mainly of water and makes up approximately 2/3 of the eye's volume, giving it form and shape. It is in contact with the retina, and helps to keep it in place.

The retina is a multi-layered sensory tissue of neural cells that line the back of the eye’s interior. It contains 3 layers of nerve cells, including the outermost layer of sensory photoreceptor cells that capture light rays and convert them into electrical impulses, which are transmitted by the optic nerve to the brain. Photoreceptors comprise two types: rods and cones. Each retina comprises approximately 125 million rods. These are responsible for peripheral vision and they function best in dim light. There are approximately 6.5 million cones in a human eye and these are more concentrated in the macula; most densely in the fovea. Cones are essential for vision in bright light and for seeing colours. The outer layer of the retina is known as the retinal pigment epithelium (RPE) layer. This layer helps to nourish the photoreceptor cells and is attached to the choroid, which provides the RPE with nourishment that includes oxygen. The innermost layer of the choroid is known as Bruch’s membrane.

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The macular is situated at roughly the centre of the retina. It is the focus for incoming light and, as such, is responsible for central vision and the ability to see detail. It has a diameter of approximately 1.5 mm.

The fovea is a small pit of around 0.3 mm near the centre of the macula, which has the highest concentration of cone cells and is free of rod cells.

The optic nerve transmits visual information in the form of electrical impulses from the retina to the brain. It connects to the back of the eye near the macula. The photoreceptor cells of the retina are not present in the optic nerve. As a result, a blind spot in the field of vision is created at the point on the retina, where the optic nerve leads back into the brain. This is not normally noticeable because the vision of one eye overlaps with that of the other.

The conjuctiva is a thin, vascularized mucus membrane that lines the inner surface of the eyelids and covers the anterior part of the sclera up to the cornea. Owing to the relative leakiness of the membrane, rich blood flow and large surface area, conjuctival uptake of a topically applied drug from tear fluid is typically an order of magnitude greater than corneal uptake [34]. Drugs absorbed by the conjunctiva appear to enter certain intraocular tissues by a mechanism which bypasses the anterior chamber [35].

2.2 Nasolachrymal drainage system [6]

The nasolachrymal drainage system (Figure 2.2) consists of a secretory, distributive and collection part. The secretory portion is composed of the lacrimal gland proper. The secreted tears are spread over the ocular surface by the eyelid during blinking. The collecting system consists of the canaliculi, the lacrimal sac, and the nasolacrimal duct, which has its opening in the inferior nasal passage. The tear production by the lachrymal gland can be subdivided into basic, reflex, and emotional tearing. The basal flow is needed to maintain a tear film on the corneal surface for optical, metabolic and lubricant purposes. The basal tear flow is of about 1.2 μl/min (range 0.5–2.2 μl/min). This results in a tear turnover rate of 16%/min during waking hours. There is no significant difference between tear production in men and women before the sixth decade, however the tear turnover rate is age-dependent, and elderly people suffer more from dry eyes. Reflex tears are induced by peripheral stimuli

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[e.g. chemical or mechanical irritation, temperature (cold), and light]. Reflex stimulation may increase lachrymation one hundred-fold, even up to 300 μl/min, resulting in wash out of a foreign body, including drugs. The absorption of lipophilic drugs can occur through the nasal mucosa during drainage, or cause adverse side effects and even toxic reactions, but the significance of reported systemic adverse effects of beta blockers is questionable.

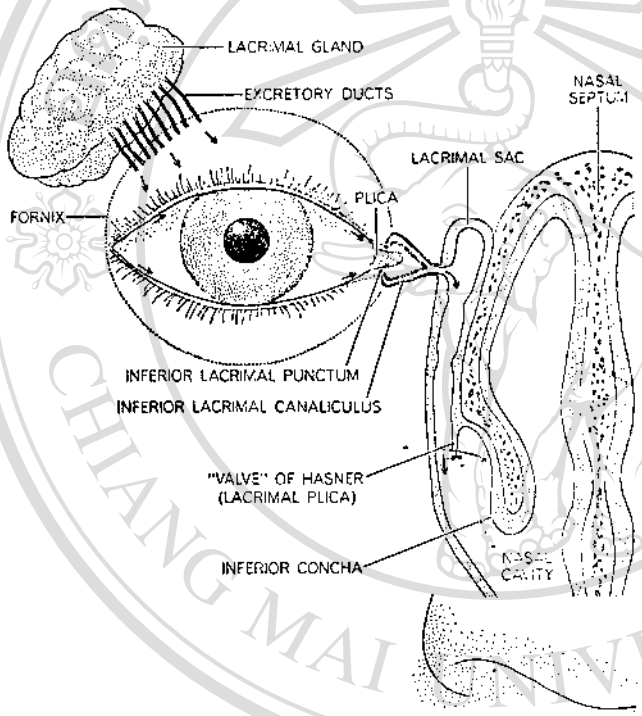

Figure 2.2 Nasolachrymal drainage system [36].

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2.3 Physiological Considerations

Successful delivery of drugs into the eye is extremely complicated because the eye is protected by a series of complex defense mechanisms, which make it difficult to achieve an effective concentration of drug within the target area [2]. After topical administration of an ophthalmic drug solution, the drug mixes with lacrimal fluid and is then diluted. Moreover, the contact time of the eye drops is the most popular and well-accepted route of administration for the treatment of various eye disorders. The bioavailability of ophthalmic drugs is, however, very poor, due to the protective

mechanisms of the eyes. The eye is a unique organ that is virtually impermeable to most environmental agents. Continuous tear flow, aided by the blink reflex, mechanically washes substances from the ocular surface and prevents the accumulation of microorganisms. In addition, lysozyme, lactoferrin, secretory immunoglobulins, and defensins are present at high levels in tears and can specifically reduce bacterial colonization of the ocular surface [37]. Moreover, the anatomy, physiology and barrier function of the cornea compromise the rapid absorption of drugs. Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage, and the high efficiency of the blood-ocular barrier [1]. Due to these physiological and anatomical, shortcomings in effect 1% or less of the instilled dose is ocularly absorbed [38].

The extent of absorption of an ophthalmic drug is severely limited by physiological contraints [3]. Among the factors that limit ocular absorption is the relatively impermeable corneal barrier. The cornea consists of three membranes, the epithelium, endothelium and inner stroma, which are the main absorptive barriers. The epithelium facing the tears with lipophilic cellular layers, acts as a barrier to ion transport. The tight junctions of the corneal epithelium serve as a selective barrier for small molecules and they prevent the diffusion of macromolecules via the paracellular route. The stroma beneath the epithelium is a highly hydrophilic layer, making up 90% of the cornea. The corneal endothelium is responsible for maintaining normal corneal hydration.

Clearly then, the more lipophilic the drugs are, the more resistance they will find crossing the stroma. The more hydrophilic a drug, the more resistant the epithelium, whereas the stroma and endothelium are limited in their resistance.

Topically applied drugs reach the bloodstream mainly via absorption across the mucosa in the nasal cavity, which is contiguous with the conjuctival sac. Consequently, delivery systems that prolong the residence time of the applied dose in the conjunctival sac would be expected to reduce systemic drug absorption. Physico-chemical drug properties such as lipophilicity, solubility, molecular size and shape, charge and degree of ionization affect the route and rate of permeation in the cornea.

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2.4 Pharmacokinetic Considerations [3]

Since the 1980s, an increase in understanding of pharmacokinetics of the eye has developed from a number of strategic clinical papers, and led to a regeneration of interest in the various formulations. Pharmacokinetic reviews have been published by Shell [39] and by Callegan et al.[13]. As before, after topical administration of an ophthalmic drug solution, the drug mixes with the lacrimal fluid. The contact time of the drug with ocular tissues is relatively short (1-2 min) because of the production of lachrymal fluid (0.5-2.2 μl/min). Then, approximately half of the drug flows through the upper canaliculus, while the other half runs through the lower canaliculus into the lacrimal sac, which opens into the nasolacrimal duct. Drainage of lachrymal fluid during blinking towards the nasolacrimal duct induces a rapid elimination of conventional dosage forms [35, 40]. The drug is absorbed into the retina-choroid via an extracorneal, or sclero-conjuctival route; the iris and ciliary body are presumably supplied via both the transcorneal and extracorneal pathways. The drug penetrates across the corneal epithelium via the transcellular or paracellular pathway. Lipophilic drugs prefer the transcellular route, while hydrophilic drugs penetrate primarily through the paracellular pathway, which involves passive or altered diffusion through intercellular spaces. The transcorneal penetration appears to be hindered by binding of the drug to the corneal tissues. The cornea may act as a drug reservoir, slowly releasing the drug into the aqueous humour, where levels decrease very slowly. Drugs are distributed from the aqueous humour to the intraocular tissues, i.e. iris-ciliary body, lens, vitreous and choroid-retina, and eliminated mainly via aqueous humour turnover and venous blood flow in the anterior uvea. It is suggested that ocular penetration via the scleroconjunctival route is more rapid (for a hydrophilic drug) than via transcorneal penetration [41]. Both transconjunctival and transnasal absorption after drainage via the nasolacrimal duct are undesirable. Transnasal drug loss causes side effects on the heart when beta-blockers are administered for the treatment of wide-angle glaucoma [42].

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2.5 New Drug Delivery Systems

Although eye drops represent 90% of all ophthalmic dosage forms, there is a significant effort directed towards new drug delivery systems for ophthalmic administration. Various new ophthalmic drug delivery systems, such as hydrogels [34], micro-and nanoparticles [43, 44], liposomes [45] and collagen shields [46-48] have been investigated. Drug delivery, as it pertains to the eye, is a generic term, which is broadly defined as representing an approach to controlling and ultimately optimizing delivery of the drug to its target tissue in the eye. An optimum ocular drug delivery system, which is therapeutically important, would be one that can be delivered in eye-drop form with no creation of blurred vision or irritancy [49]. The benefits to the patient would be simplicity, a diminished frequency of administration, lower toxicity, and no undesirable side effects. The emergence of new and innovative means for improving therapeutic efficacy suggests that a greater choice of dosage forms will be provided to physicians and patients in the next decade. Most of the formulation efforts aim at maximizing ocular drug absorption through prolongation of the drug residence time in the cornea and conjunctival sac, as well as slowing drug release from the delivery system and minimizing precorneal drug loss. To overcome these problems, various ophthalmic vehicles such as solutions, suspensions, ointments, inserts, and aqueous gels, have been investigated to extend the ocular residence time of medications for topical application to the eye.

2.6 Chitosan

Chitosan is a deacetylated derivative of chitin, a natural polymer. It is widely used as a pharmaceutical excipient [50]. Chitosan comprises a series of polymers varying in their degree of deacetylation, molecular weight, viscosity, pKa, etc, and has a cation intensified contact with the mucosa. The presence of a number of amino acids permits chitosan to chemically react with anionic systems, thereby resulting in alteration of the physicochemical characteristics of such combinations. Due to high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity enhancing agent in an acidic environment. It behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solution rises with an increase in chitosan concentration, and decreases in

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temperature with on increasing degree of deacetylation. Chitosan has several favorable biological properties such as a penetration enhancing, drug loading and concentration gradient increasing effect, bio-degradability, non-toxicity, bio-compatibility and excellent ocular tolerance. Topical use of chitosan in ophthalmology tolerance assessment and evaluation of precorneal retention has shown that it increases corneal residence 3-fold in time when compared to Tobrex^{TM} [22]. These characteristics make it very attractive for ophthalmic formulations.

Chitosan as an active biomaterial in ophthalmology

Chitosan is a very interesting biomaterial in ophthalmology not only because of its favourable biological properties as indicated above, but also due to its inherent biological activity, which may also have an impact on ocular therapeutics.

The various forms in which chitosan has been investigated in ophthalmology are indicated in Table 2.1.

Besides being a major component in drug delivery devices, chitosan itself has been shown to have wound healing and antibacterial activity [51, 52]. These effects could be very beneficial for the treatment of a number of ocular diseases such as bacterial keratitis. The idea of using chitosan in corneal wound healing came from a published research which showed that chitosan accelerated wound-healing [53, 54]. More precisely, based on this previous work, it was hoped that chitosan would play an active role by increasing keratocyte migration, thereby leading to a more rapid production of collagen and improving corneal healing. Felt et al. [21] found that chitosan solutions containing 0.5% w/v of a low molecular weight (Mw) chitosan (160 kDa) were assessed for antibacterial efficacy against *E. coli* and *S. aureus* by using the usual broth-dilution technique. Besides the bacteriostatic activity of chitosan, Felt et al. [22] studied that the mucoadhesive polysaccharide chitosan increased precorneal drug residence times.

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Table 2.1 Forms of chitosan investigated in ophthalmology (modified from Alonso and Sanchez [17])

Chitosan form	Application	Drug incorporate	Reference
Contact lenses	Corneal wound healing	-	[55]
Solution	Dry eye	-	[21, 56]
Solution	Prolonged retention	Tobramycin	[19]
Liposomes	Evaluated for the in vivo uptake and acute tolerance of the nanosystems	-	[57]
Liposomes	Long-lasting	Pilocarpine-loaded chitosan/Carbopol nanoparticles	[58]
Microspheres	Improve corneal penetration	Acyclovir	[59]
Microspheres	Improved corneal penetration	Ofloxacin	[60]
Nanoparticles	Long-term drug level	Indomethacin	[61]
Nanoparticles	Improved corneal penetration	Cyclosporin	[62]

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The residence time of topical eye drops refers to the duration of its contact with the ocular surface. This concept is of particular interest in the formulation of topical ocular drug vehicles, where mucoadhesive polymers are frequently used as an approach to prolong drug residence times. When using a mucoadhesive material, the clearance of the drug is controlled by the mucus turnover rate, which is much slower than the tear turnover rate. This prolonged retention of the drug formulation implies, for a drug with good permeability properties, an enhanced ocular drug bioavailability.

Chitosan is in this category of mucoadhesive polymers. The mucoadhesive character of chitosan relates to the attraction between its positively charged amino groups and the negatively charged residues of sialic acid in the mucus [18] along with other forces such as hydrogen bonds [63]. In addition to this special property, chitosan exhibits other attractive features, mentioned in the following paragraphs, which make it a unique candidate for ocular drug delivery. The penetration-enhancing properties of chitosan were initially attributed to the modulation of the tight junction barrier between epithelial cells [64] and they were also related to intracellular routes [17]. Chitosan is biodegradable [17, 65], which enables its safe administration and degradation of topically applied ocular chitosan vehicles. As mentioned before, chitosan biodegradation is mediated by the hydrolytic actions of lysozyme and other enzymes (i.e. human chitinase and N-acetyl-β-D-glucosaminidases), which produce chito-oligomers and monomers [17]. This susceptibility to enzymatic depolymerization is an exclusive characteristic of chitosan, with respect to other polysaccharides. The degradation rate in the presence of lysozyme depends on the degree of acetylation. Chitosan has excellent ocular tolerance. This has been reported in a rabbit model following topical application of chitosan solutions and using confocal laser scanning ophthalmoscopy combined with corneal fluorescein staining [22]. Chitosan has favourable rheological behaviour, and its solution has shown pseudoplastic and viscoelastic properties [66]. These properties are very important characteristics, since the pre-corneal tear film has a pseudoplastic character that should not be disturbed by application of liquid formulations. On the other hand, viscoelastic fluids exhibit high viscosity under low shear rate and low viscosity under high shear rate conditions. This behaviour is particularly important in ophthalmic formulations, since it facilitates the retention while permiting easy spreading of the formulation, due to blinking of the eyelids. On the basis of these favourable biological properties, and also because of its adaptability for designing different delivery systems, chitosan has attracted great attention in the pharmaceutical and biomedical fields. Nevertheless, the number of reports on the potential of the cationic polysaccharide in the ophthalmic field is still limited [20]. The following experiment described the characteristics of chitosan and the *in vitro* and *in vivo* behaviour of

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chitosan-based ocular drug delivery for vancomycin. Vancomycin eye drops is a drug of choice for bacterial keratitis.

2.7 Bacterial keratitis

Microbial keratitis is a major cause of monocular blindness in developing countries [67, 68]. In Thailand, Jenchitr [69] demonstrated that corneal ulcers were the second most common cause of blindness. Micro-organisms isolated from corneal specimens are more bacterial than fungal (Table 2.2) [68]. Bacterial keratitis listed as common pathogenic organisms at Maharaj Nakorn Chiang Mai Hospital are shown in Table 2.2 [68]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most important, followed by *Streptococcus pneumoniae* and *Moraxella*. In an individual case, however, any host of gram-positive or negative bacteria, including anaerobes, may be involved; mixed infection is not uncommon. Under normal conditions, the cornea is protected from invasion by its epithelium and the defensive mechanisms of the external eye. Only a few bacteria are able to penetrate intact corneal epithelium (*Neisseria, Corynebacterium diphtheriae*, *Haemophilus*, and *Listeria*). Therefore, epithelial injury is an important factor in the development of corneal ulcers. Risk factors for corneal ulcers and typical organisms involved are shown in Table 2.3 [70]. Other predisposing and precipitating factors are listed in Table 2.4 [68].

2.7.1 Some common ocular pathogens [71]

2.7.1.1 Gram-positive cocci

A. Staphylococci

Some staphylococci are normal inhabitants of the skin and mucous membranes, whereas other species are capable of producing conditions such as boils, abscesses and even a fatal septicaemia. Others can cause food poisoning by the liberation of an enterotoxin. Resistance to certain antibiotics develops easily; the term ‘hospital Staph’ used to be applied to some resistant forms, but the modern term is MRSA (methicillin-resistant *Staphylococcus aureus*). Staphylococci are differentiated from streptococci by the presence of an enzyme that breaks down hydrogen peroxide (catalase). Pathogenic staphylococci possess coagulase, which clots blood plasma.

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Table 2.2 Micro-organisms isolated from corneal specimens

Bacterial	Number of eyes (%)
Gram positive cocci	
Streptococcus pneumoniae	4 (7.02)
Streptococcus viridian	2 (3.50)
Staphylococcus aureus	2 (3.50)
Coagulase negative staphylococcus	1 (1.75)
Gram negative bacilli	
Pseudomonas aeruginosa	22 (38.60)
Serratia marcescens	1 (1.75)
Gram negative cocci	
Moraxella spp	1 (1.75)
Norcadia	1 (1.75)
Mixed P.*aerusginosa* and *S.sapriphyticus*	1 (1.75)
Total	35 (61.37)
Fungal	**Number of eyes (%)**
Fusarium spp	8 (14.04)
Aspergillus spp	4 (7.02)
Cuvularia spp	2 (3.51)
Paecilomyces spp	1 (1.75)
Cladosporium spp	1(1.75)
Exophiala spp	1 (1.75)
Trichosporon spp	1 (1.75)
Citrobactor freundii	1(1.75)
Unidentified septate hyphae	1 (1.75)
Candida albican	1(1.75)
Pythium	1(1.75)
Total	22 (38.6)

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Table 2.3 Risk factors for corneal ulcers and typical organisms involved

Factor	Typical organisms
External:	
Contact lenses: soft; extended wear; disposable and overnight wear (highest risk); aphakic	*Pseudomonas and staphylococci*
Corticosteroid eye drops	*Strep. pneumoniae*
Other eye drops, cosmetics	*Pseudomonas*
Warm, humid climate	*Pseudomonas*, fungi
Intensive care units, respirators	*Pseudomonas*
Local:	
Dry eye states	-
Bacterial or fungal conjunctivitis	-
Conjunctival shrinkage	Gram ± ve bacteria
Lagophthalmus,neuroparalytic keratopathy	-
Persistent epithelial defects	-
Bullous leratopathy	-
Herpetic ulcers	-
Trauma, contact with plant material	Fungi
Corneal surgery	-
Dacryocystitis	*Strep. Pneumoniae*
Entropion, trichiasis	-
Systemic:	
Poor nutrition	-
Alcoholism	-
General debility	-
Immunosuppression	Protozoa, HSV, HZV
Diabetes	*Moraxella*

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Table 2.4 Frequency of predisposing risk factors in microbial keratitis

Predisposing factors	Number of cases (%) N=214
Trauma	95 (44.39)
Injury	39 (18.23)
Foreign body	54 (25.23)
Surgery	2 (0.93)
Ocular surface disease	31 (14.48)
Corneal disease	20 (9.34)
Lid/lash abnormalities	11 (5.14)
Contact lens wear	32 (10.75)
Steroid use	8 (3.74)
Underlying systemic disease	19 (8.88)
None	38 (17.76)

In the eyes, staphylococci can cause infections of the lids, lacrimal apparatus, conjunctiva and cornea. Infections of the lash follicle can result in the formation of a stye (hordeolum). Because *Staph. aureus* is so common, it is often employed in the efficiency testing of preservative systems. *Staphylococcus epidermidis* is usually considered to be a commensal and normal inhabitant of the skin. Unlike *Staph. aureus*, it produces white colonies. Maske et al. [72] found a higher than normal incidence of *Staph. epidermidis* in a group of patients with bacterial corneal ulcers. It releases a toxin that causes some signs of blepharitis and keratopathy.

B. Streptococci

Streptococci lack the enzyme catalase and are characterized by their ability to cause haemolysis. Complete haemolysis is brought about by beta-haemolytic streptococci; the haemolysis produced by alpha-haemolytic species is incomplete and leads to the formation of green pigment. There are also non-haemolytic streptococci.

Streptococci can produce local and general infections. One of the most common local infections of beta-haemolytic streptococci is the

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streptococcal sore throat, which can extend to the middle ear in young children and cause otitis media. On the skin, they can cause impetigo. Beta-haemolytic streptococci infections give rise to puerperal fever, wound sepsis and encarditis. It is fortunate that penicillin continues to be effective against many strains of streptococci.

In the eye, streptococci can cause conjunctivitis, dacryoadenitis, dacryocystitis and blepharitis. Jones et al. [73] reported corneal ulcers, endophthalmitis, conjunctivitis and dacryocystitis resulting from streptococcal infections. The ability of streptococci to cause sight-threatening infections is of concern because many strains are not susceptible to gentamicin, an antibiotic often chosen to treat such infections.

2.7.1.2 Gram-negative cocci

The Neisseriae is a group of Gram-negative bacteria, which include the normal flora of the respiratory system and pathogens that cause meningitis (*Neisseria meningitidis*), and gonorrhoea (*Neisseria gonorrhoeae*). In the eye, *Neisseriae* species can infect the lids, lacrimal apparatus and conjunctiva, and *N. gonorrhoea* is best known as the one-time principal cause of ophthalmia neonatorum, an infection that occurs as the infant passes down the birth canal. The disease manifests between the second and fifth day after birth, when the lids become swollen and there is a bilateral purulent discharge. The lids are tightly closed and difficult to open and the acute phase lasts for 4-6 weeks. The condition is treated with topical and systemic antibacterials. If treatment is not carried out, the cornea can become involved and the eye lost. However, other causative organisms, such as *Chlamydia* species, and other causes (paradoxically, the overenthusiastic use of silver nitrate) are more important today.

2.7.1.3 Gram-positive rods

A. Corynebacterium diphtheriae

Corynebacteria are non-motile Gram-positive rods; they do not form spores. Some species are normally resident in the human respiratory tract. *Corynebacterium diphtheriae,* when infected with the appropriate bacteriophage, produces a powerful exotoxin that causes diphtheria. This disease results in the growth of a membrane across the throat, leading to suffocation. It can similarly affect the eyelids, with the appearance of such membranes on the inner surface of the lids.

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The conjunctiva can become involved in the same way. Diphtheroids have been isolated in a proportion of infected conjunctivae.

B. Clostridium species

The Clostridia are a group of obligate anaerobes notorious for their pathogenicity. In particular, they include *Clostridium botulinum*, which, when infecting food, produces botulinum toxin. Although botulinum toxin ingestion is potentially pathogenic, this substance has been used to paralyse the antagonist muscles in cases of paralytic strabismus and other ocular disorders. *Clostridium tertani* is a possible infectant of deep wounds and routine prophylaxis against the effects of its toxin. *Clostridium welchii* and *Clostridium oedematiens* cause gangrene. Gas gangrene of the lids has been reported [74].

2.7.1.4 Gram-negative rods

A. Pseudomonas aeruginosa

Pseudomonas aeruginosa is perhaps the most notorious bacterium for causing ocular problems and is normally found in small numbers in the gut and on the skin. It is a common contaminant of water and has been cultured from jacuzzis [75]. Its numbers are kept in check by the presence of other organisms, but, as it is resistant to many antibiotics, it can gain dominance if the surrounding organisms are suppressed. *Ps. aeruginosa* produces a bluish green colour when grown on media. It has a characteristic odour and is pyogenic; the presence of green pus suggesting pseudomonal infection.

Ps. aeruginosa is an opportunistic organism normally kept at bay by the body's defence mechanisms. If these are breached, a serious infection often results, *Ps. aeruginosa* can infect burns, especially over a large area, and can also gain hold in patients who are immune-compromised. *Ps. aeruginosa* is an extremely versatile organism in that it can metabolize fluorescein and hydroxybenzoates as carbon sources for energy, which means that it can survive in conditions that are alien to most other organisms. The organism is susceptible to antibiotics such as gentamicin and polymixin.

In the eye, *Ps.aeruginosa* can produce meibomitis, conjunctivitis and corneal ulcers and is one of the causes of ophthalmia neonatorum [76]. Should access be gained to the sterile interior of the eye, then panophthalmitis

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might result and, indeed, has been responsible for causing more than one serious case of hospital acquired disease leading to the loss of an eye. *Ps. aeruginosa* is an important test organism for contact lens solutions and eyedrop preservative systems, not only for its virulence when an infection is established, but also because of its biochemical versatility, which sometimes makes it difficult to eradicate.

B. Haemophilus species

These small, aerobic organisms get their name from their requirement for enriched media containing blood for culturing *in vitro*. They include certain important human pathogenic organisms. *Haemophilus influezae* is a secondary invader, which helps to produce some of the symptoms of influenza and can bring about inflammation in most parts of the respiratory tract. *Bordetella pertussis* is another member of this group that affects the respiratory system, causing whooping cough, which is transmitted by airborne infections from one person to another. *B. pertussis* cannot exist for long periods outside the body. Similarly, *Haemophilus ducreyi* is so fastidious in its requirements that it can only be transmitted sexually and, consequently, is the causative organism of chancroid, a form of veneral disease.

H. influenzae and *H. ducreyi* can infect ocular tissues. Two members of this group, however, are particularly noted for their ability to cause conjunctivitis: *Haemophilus aegyptius* (*Haemophilus conjunctivitidis*, Koch-Weeks bacillus) is often the cause of acute epidemic conjunctivitis, especially in school-children, and *Moraxella lacunata* (Morax-Axenfeld bacillus) is another well-known causative organism of conjunctivitis.

2.7.2 Clinical features of bacterial keratitis

In a typical case of bacterial keratitis, symptoms include pain, photophobia, conjunctival hyperemia, and epiphora. Signs include discharge, blepharospasm, lid swelling, and reduction in visual acuity. Corneal ulcers may be of differing shape, depth, and localization. Gram-positive organisms often cause sharply demarcated round or oval infiltrates, while gram-negative ones tend to generate rapidly progressive diffuse infiltrates with mucinous necrosis. Usually, there is an anterior chamber reaction; sometimes, frank hypopyon. Some pathogens (*Pseudomona*s, staphylococci, and streptococci) produce a typical clinical appearance,

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permitting tentative diagnosis. In any case of corneal ulcer, the physician must decide whether it is of infectious origin usually associated with an epithelial defect.

2.7.3 Treatment

Corneal ulcers constitute an ophthalmological emergency, particularly if they are centrally situated and have deep margins. Treatment must commence as soon as specimens have been collected for microbiological diagnosis. For severe cases, or those not responding to therapy, inpatient management should be considered.

In the case of a Gram stain being available, treatment can be guided by the result. It should, however, still cover a broad spectrum until the results of culture and sensitivity testing are known. When streptococci or staphylococci appears on a gram-stained smear, bacitracin, cefazolin, or vancomycin is recommened. Gram-negative pathogens are treated with aminoglycosides (gentamicin, amikacin, tobramycin), ceftazidime or cefuroxime. The administration of a single broad-spectrum antibiotic, either 0.3% norfloxacin, ofloxacin, or ciprofloxacin (FDA-approved for this indication), instilled hourly around the clock, is commonly used. While relatively convenient for the patient, this does not cover the full spectrum quite as well as two complemetary antibiotics. Additionally, it may foster the development of resistance. The efficacy of ofloxacin solution in treating bacterial keratitis is equivalent to that of the fortified cefazolin and tobramycin solutions [77]. The reduced frequency of ocular toxic effects and the relative ease of preparing of ofloxacin are additional considerations. In severe cases, appropriate initial therapy is most critical in the course of serious corneal ulcers, and aggressive, broad-spectrum antibiotic coverage is advocated [78]. The physician should consider using fortified antibiotic medications [32, 70, 79, 80] (Table 2.5).

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Table 2.5 Preparing fortified topical antibiotics [33]

Antibiotic	Commercially available concentration or amount	Volume of diluting fluid (ml)	Type of diluting fluid	Therapeutic concentration (%)
Amikacin	50 mg/ml	9	water for injection	25 mg/ml
Ampicillin	500 mg	9.5	water for injection	50 mg/ml
Bacitracin	50,000 IU	9.8	water for injection	5,000 IU/ml
Cefazolin	500 mg	4.8 to 9.8	0.9% sodium chloride	50 to100 mg/ml
Gentamicin	80 mg/2 ml	5	gentamicin ophthalmic solution 3 mg/ml	13.6 mg/ml
Vancomycin	500 mg	10	artificial tear, 0.9% sodium chloride	50 mg/ml

2.7.3.1 Routes of administration to the eye

- Topical, either as liquid, ointment, or solid insert
- Periocular injection, including subconjuctival, sub-Tenon's, or retrobulbar
- Intraocular injection
- Systemic, including intravenous, intramuscular, or oral route.

2.7.3.2 Dosage forms [33]

There are four main routes of ophthalmic drug administration, and specific products for these routes. Topical application can be achieved through the use of liquid drops, viscous ointments, or solid inserts.

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2.7.3.3 Preparations for topical administration [33]

Topical administration is the most common and convenient route for ocular drug delivery. The factors determining a product's feasibility are the drug's aqueous solubility, its stability as a liquid, and the dosage form in which it is commercially available.

Drugs which are not water soluble may be formulated as suspensions for topical use, but not as extemporaneously prepared injections. Substances that are only stable for a few hours after reconstitution are not practical for topical use because of the necessity for short expiration dating. Drugs that are available only as oral dosage forms present serious difficulties in reformulation as ocular products.

Extemporaneous eye drop solutions for topical ocular administration are the simplest formulations for allowing high drug concentrations contact with the eye. Many commercially available parenteral drugs are readily made into aqueous solutions suitable for use as eye drops. The outer surface of the eye will tolerate preparations with pH values of between 3.5 and 10.5; however, products outside the pH range of 6.5 to 8.5 may cause corneal damage if contact is prolonged.

It is not necessary for eye drops to be isotonic. If there is a prolonged contact time between the drop and the eye, as occurs with the use of viscous eye drops, isotonicity becomes important. Hypotonic drops may cause corneal edema, while hypertonic drops will be painful on instillation, due to their temporary dehydrating effect on the corneal epithelium.

The two major components of a topical formulation are the drug and the vehicle. Preferably, the drug to be formulated should be available as a sterile powder or solution for parenteral injection. If not available in an injectable dosage form, preparation of a topical product is more difficult.

Commercially available artificial tears are often acceptable vehicles for topical drop formulations. Artificial tears contain emollients and a buffer system for eye comfort, and viscosity agents for more prolonged contact time. These are commercially available in ophthalmic dropper bottles, which can be used to hold the final product.

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Unfortunately, the use of tear solutions has three major disadvantages. Firstly, federal law mandates that multiple dose containers of ophthalmic products be formulated with suitable preservatives to prevent microbial contamination while the product is in use. When used at the recommended frequencies or on damaged corneal tissue, further damage to the corneal and/or delays in its healing may result.

The second disadvantage in the use of artificial tear products for extemporaneous vehicles is the dilution of the tear's preservative(s). The addition of this unpreserved volume to a preserved tear product necessarily reduces the total concentration of the preservatives. The result of such dilution on preservative effectiveness is unclear, but it can be substantial. The use of an artificial tear product for all dilutions (rather than sterile water or saline) in a formulation eliminates this problem, and is used as an acceptable method in some eye specialty institutions.

Finally the preservative included in artificial tear products (typically benzalkonium chloride) may produce unknown problems when mixed with the preservatives (often paraben compounds) of the parenteral drug in the final product. It is the unknown elements in these arguments that speak against the routine use of artificial tear products as topical eyedrop vehicles.

In the use of preservatives, most ophthalmic specialty pharmacists believe that extemporaneous topical eye preparations should be formulated with unpreserved vehicles whenever possible. For practical purposes, the vehicle of choice is unpreserved sterile water or saline for injection. While avoiding preservative toxicity, the unpreserved formulations are commonly discarded after 24 hours of use, to avoid microbial contamination. This creates the need for fresh solution preparation on a daily basis, a situation that is only feasible in a hospital inpatient setting. Additionally, because these sterile water or saline solutions have no buffers, their pHs may be outside the optimal ocular range (pH 6 to 8). Although human tears have some buffering capacity, they may not be able to buffer a drug product sufficiently to make it comfortable for the patient.

In many institutions, the problem of preserved versus unpreserved topical formulations is solved in the following manner. For patients who are hospitalized for treatment of their ocular infections, antimicrobial agents are

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formulated without preservative, and a fresh supply is provided daily. At the time of discharge from the hospital, when it may be assumed that significant healing has occurred, the vehicle is changed to bacteriostatic water/saline for injection, and the dating on the product is extended, based upon available stability information on the drug at that concentration.

2.8 Vancomycin

Although vancomycin is an antibacterial glycopeptide unrelated to penicillin, it produces its antibacterial effect by interfering with cell wall production [71]. This bactericidal antibiotic is highly effective against staphylococci that have become resistant to all other drugs [70]. Its spectrum includes nearly all gram-positive organisms. Although it penetrates well into the eye, effective concentrations cannot be obtained in the vitreous of the normal eye by means of parenteral administration. In aphakic and/or viterectomized eyes, however, the minimum inhibition concentration (MIC) for common gram-positive organisms can be exceeded.

In combination with an aminoglycoside or a ceftazidime, vancomycin is the drug of choice for intravitreal injection in endophthalmitis (1 mg). It can be used at a concentration of 50 to 100 μg/ml in the irrigation fluid employed during cataract surgery, and in concentrations of 30 μg/ml during vitrectomies. One milligram and more of vancomycin can be safely injected into the anterior or posterior chamber.

Topical use (25 to 50 mg/ml) for bacterial keratitis caused by gram-positive organisms is very effective, but often toxic due to the low pH unless a buffer is used. Inhibitory concentrations can be achieved within the anterior chamber.

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Vancomycin should not be used indiscriminately, as defense against multiresistant staphylococci may result if resistance to vancomycin develops.

In a systemic dosage of 500 mg bid to qid i.v.; lower doses must be employed where renal function is impaired. Oral vancomycin, in a dose of 150 to 200 mg qid, is used for *Clostridium difficile* colitis. It is not absorbed to any extent.

The side effects of i.v. administered vancomycin are ototoxic and nephrotoxic, especially when peak serum levels exceed 30 to 40 μg/ml. Other adverse effects are skin reactions, fever, flushing, and pruritus. Necrosis of the conjuntiva prevents effective dosing in subconjuctival injections (25 mg).

Successful therapy for bacterial keratitis must be able to rapidly attain drug concentrations at the site of infection. Since the cornea is not vascularized, it is not readily permeated by systemically administered drugs, which are, therefore, not used for the treatment of keratitis. On the other hand, topical treatment may fail to achieve therapeutically active drug levels in the cornea, as continuous tear flow reduces the bioavailability of topically applied antibiotic, and the corneal epithelium acts as a barrier against drug penetration. For this reason, standard treatment of severe bacterial keratitis requires administration at frequent intervals (every 15 to 60 minutes for 48 to 72 hours) of eye drops containing fortified solution (those more concentrated than commercially available ones) of fluoroquinolones or multiple antibiotics, usually a cephalosporin, an aminoglycoside and glycopeptides [12, 14, 30, 81-83]. However, this regimen is not only disruptive to the patient and usually necessitates hospitalization, but it has also been associated with *in vitro* toxicity to the corneal epithelium. Efforts are now directed to testing new antimicrobials that permeate the cornea better and develop systems capable of prolonging the contact time between antibiotics and the corneal tissue, thereby, potentially enhancing intracorneal delivery of ophthalmic medication.

Chitosan, cationic polymer, is biodegradable, biocompatible and non-toxic. Chitosan is in this category of mucoadhesive polymers. When using mucoadhesive material, the clearance of the drug is controlled by the mucus turnover rate, which is much slower than the tear turnover rate. This prolonged retention of the drug formulation implies an enhanced ocular drug bioavailability for a drug with good permeability properties [17, 22]. Chitosan is a very promising biomaterial in ophthalmology not only due to the favourable biological properties indicated above, but also because of its inherent biological activity, which may also have an impact on ocular therapeutics.

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