Polymeric Hydrogels for Controlled Drug Delivery to the Eye

Indrajeetsinh B. Sarvaiya

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Biomaterials and Drug Delivery Group
School of Pharmacy and Biomedical Sciences
University of Portsmouth

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Declaration

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Indrajeetsinh Sarvaiya

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This project has been directed towards the design, preparation and evaluation of novel polymeric vehicles that can control the release of ophthalmic drugs.

Hydrogels based on several synthetic and naturally-derived polymeric and monomeric units, including poly(vinylpyrrolidone-co-[meth]acrylic acid)s, N-isopropylacrylamide or 2-hydroxyethyl methacrylate and different types of chitosan, were synthesised, and the effects of hydrogels' network structure and composition upon their swelling properties, adhesion behaviour and drug release characteristics were examined.

These novel materials were formulated as either ophthalmic inserts or as nanosuspensions, and were investigated both in vitro and in vivo for their ability to act as controlled release vehicles for ophthalmic drug delivery.

Comparative in vitro studies employing a range of common ophthalmic drugs (chloramphenicol, atropine, norfloxacin or pilocarpine) informed the selection of drug-specific carrier compositions for the controlled delivery of these compounds, and indicated that some of these materials have a high potential for the delivery of sparingly water-soluble actives.

In vivo (rabbit model) experiments involving the delivery of pilocarpine showed that chitosan-based hybrid polymeric networks containing 2-hydroxyethyl methacrylate may be potentially useful carriers for the delivery of this therapeutic agent.
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List of abbreviations

Ø: diameter

\(^{14}\)C-AA: \(^{14}\)C-radiolabelled acrylic acid

AA: acrylic acid

ACVA: 4,4'-azobis(4-cyanovaleric acid)

ATP: atropine sulphate

AUC: area under curve (miosis-time profile)

CHF: chloramphenicol

cp(VP/AA): linear poly(vinylpyrrolidone-co-acrylic acid) copolymers

cp(VP/AA)*: linear poly(vinylpyrrolidone-co-\(^{14}\)C-acrylic acid) copolymer

cp(VP/AA)M: poly(vinylpyrrolidone-co-acrylic acid) copolymers crosslinked with \(N,N'\)-methylenebisacrylamide

cp(VP/AA)M_1*: radiolabelled poly(vinylpyrrolidone-co-\(^{14}\)C-acrylic acid) copolymer crosslinked with \(N,N'\)-methylenebisacrylamide

cp(VP/MA): linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers

cp(VP/MA)M: poly(vinylpyrrolidone-co-methacrylic acid) copolymers crosslinked with \(N,N'\)-methylenebisacrylamide

CS: commercial low molecular weight chitosan

CSf: acrylic acid-functionalised chitosan

CSo: commercial chitosan oligosaccharide lactate

CSo/cp(VP/MA): crosslinked hydrogels based on chitosan oligosaccharide lactate and poly(vinylpyrrolidone-co-methacrylic acid) linear copolymers

CSo/PMA: crosslinked hydrogel based on chitosan oligosaccharide lactate and poly(methacrylic acid) linear polymer

DA: degree of deacetylation

DS: degree of swelling
DS_{eq}: equilibrium degree of swelling
EDAC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
HEMA: 2-hydroxyethyl methacrylate
HPN: hybrid polymeric networks
hpn(CSf/HEMA): hybrid polymeric networks based on acrylic acid-functionalised chitosan and hydroxyethyl methacrylate
hpn(CSf/NIPAM): hybrid polymeric networks based on acrylic acid-functionalised chitosan and N-isopropylacrylamide
Hq: hydroquinone
ic(CS/PHEMA): interpolymeric complexes of low molecular weight chitosan with poly(N-isopropylacrylamide)
ic(CS/PNIPAM): interpolymeric complexes of low molecular weight chitosan with poly(hydroxyethyl methacrylate)
IPN: interpenetrating polymeric networks
MA: methacrylic acid
MBA: N,N'-methylenebisacrylamide
MDF: maximum detachment force
MIC: minimum inhibitory concentration
MP: maximum pupillary response
n_{exp}: number of experiments
NFX: norfloxacin
NHS: N-hydroxysuccinimide
NIPAM: N-isopropylacrylamide
np(CS0/cp(VP/MA)): nanoparticles of crosslinked hydrogel based on chitosan oligosaccharide lactate and poly(vinylpyrrolidone-co-methacrylic acid) copolymers
np(CSo/cp(VP/AA))*: $^{14}$C-radiolabelled nanoparticles of crosslinked hydrogel based on chitosan oligosaccharide lactate and poly(vinylpyrrolidone-co-acrylic acid) copolymers

PAA: poly(acrylic acid)

PBS: phosphate buffer solution

PEO: poly(ethylene oxide)

PMA: poly(methacrylic acid)

PVP: poly(vinylpyrrolidone)

p(VP)M: poly(vinylpyrrolidone) polymers crosslinked with $N,N'$-methylenebisacrylamide

SD: standard deviation

THF: tetrahydrofuran

TWA: total work of adhesion

VP: 1-vinylpyrrolidin-2-one (vinylpyrrolidone)

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**Explanatory notes regarding the abbreviations used for the polymeric materials prepared in this work**

prefix indicating the type of material and/or formulation (e.g. cp = copolymer; hpn = hybrid polymeric network; ic = interpolymeric complex; np = nanoparticles)

$X(Y/Z)M_n^*$

indicates the sample number

indicates that the material has been crosslinked with MBA ($N,N'$-methylenebisacrylamide)

indicates that the material contains $^{14}$C radiolabel

indicates the chemical composition (e.g. VP/AA = vinylpyrrolidone and acrylic acid)

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*e.g. cp(VP/AA)M_71 =* copolymer prepared from vinylpyrrolidone and acrylic acid, crosslinked with $N,N'$-methylenebisacrylamide, sample no. 71

*ic(CS/PHEMA)_2 =* interpolymeric complex prepared from low molecular weight chitosan and poly(N-isopropylacrylamide), sample no. 2
Chapter 1 - Introduction

1.1. Challenges faced by drug delivery to the eye

In most cases, topical drug delivery is the preferred route for the treatment of ocular diseases and associated infections. The ophthalmic delivery of drugs (β-blockers, cholinergic or antibacterial agents) has been adapted from existing systemic formulations, taking little account of the intrinsic physiology of the eye.

The main barrier to efficient ophthalmic drug delivery is the rapid elimination of conventional liquid drops from the eye. Instilled eye drops are typically 30-75 μl in volume, a portion of which is quickly drained, until the precorneal region contains the normal resident volume of around 7 μl [1]. The turnover of lachrymal fluid (approximately 0.16 μl/minute) affects the concentration of the drug remaining on the surface of the eye. As the precorneal fluid is replaced by washing with lachrymal fluid, so the residual drug concentration is reduced. The concentration of drug in the precorneal area acts as a driving force for its transport through the cornea. If the precorneal drug concentration falls below that of the corneal epithelium, uptake of drug into the cornea ceases [2]. Several factors lead to a high rate of lacrimal drainage, paramount amongst which are: the rapid tear turnover and the ensuing precorneal loss, and the induction of tear flow due to irritation caused by chemical entities within the drug preparation and/or by the relatively large volume of the administered eye drops. The precorneal half-life of the active in a typical ocular formulation is considered to be of the order of 1-3 minutes [3]. As a consequence, only a very small proportion (1-3%) of the therapeutic agent penetrates the cornea and has
the opportunity to reach intraocular tissues [4]. Furthermore, the posterior segments of the eye are protected with refinement from the external environment, and this poses unique and fairly challenging obstacles for drug delivery [5].

In the last decades considerable research effort has been directed towards the improvement of ophthalmic drug delivery. The various approaches attempted in the early stages can be divided into two main categories: bioavailability improvement, and controlled-release drug delivery. The former was attempted using methods that aim to improve the corneal penetration of the drug molecule and to delay its elimination from the eye by means of viscosity enhancers, gels, nanosuspensions, emulsions, penetration enhancers, prodrugs, or liposomes [4-7]. Controlled release delivery was attempted by using various types of erodible or soluble inserts, by utilising hydrophilic or water insoluble inserts or by employing membrane-controlled diffusional inserts [8].

Although successful in prolonging the contact time between drugs and the corneal surface, many of these systems have been associated with problems of discomfort and blurring of the vision in patients, resulting in poor patient compliance. Non-mucoadhesive polymers are quickly cleared from the eye and may blur the vision [9]. Mucoadhesive polymers can lead to matted eyelids after sleep but these are reported to blur vision to a lesser extent than ointments [10]. In situ gelling systems often require a high polymer concentration and may cause irritation to the patient. Inserts are not user-friendly and suffer with poor patient compliance [2]. Liposomes have been shown to exhibit prolonged association with the external tissues of the eye but not necessarily with the cornea, and nanoparticles have presented problems with controlling the release rate of drugs [11]. Thus, there remains the need for an ophthalmic delivery system that prolongs the contact time between the drug of choice and the corneal epithelium, and affords controlled release of the drug into the precorneal area as well as comfort and ease of use to the patient.
To explain the context of this work, and to clarify some technical terms, the next sections present some details of the anatomy and physiology of the eye, common ophthalmic diseases and associated medications. Drug delivery to the eye is discussed then in more detail, and examples of current ophthalmic delivery systems, either in use or still under study, are given. A special attention is paid to ocular inserts and to mucoadhesivity, an important characteristic of the delivery formulation that can prolong the contact time between the active and the corneal epithelium, with a dramatic increase of the bioavailability of the active. Finally, brief reviews of each of the main physical methods used in this study are presented.

1.2. Anatomy and physiology of the eye

The eye is the organ of the sense of sight that is situated in the orbital cavity and it is supplied by the 2\textsuperscript{nd} cranial nerve (optic nerve). It is almost spherical in shape and is about 2.5 cm in diameter. The space between the eye and the orbital cavity is occupied by adipose tissue. The bony walls of the orbit and the fatty layer help to protect the eye from injury. The two eyes are structurally separate but unlike the ear some of their activities are coordinated so that they can function as a pair. It is possible to see with only one eye but three-dimensional vision is impaired when only one eye is used, especially in relation to the judgement of distance.

There are three main layers of tissue in the walls of the eye.

- The outer fibrous layer: sclera and cornea
- The middle vascular layer or uveal tract: choroids, ciliary body and iris
- The inner nervous tissue layer: retina
Inside the eyeball there are other structures such as the lens, aqueous fluid (humour) and vitreous body (humour).

![Diagram of the eye](image)

Sclera and cornea

The **sclera**, or white of the eyes, forms the outermost layer of tissue of the posterior and lateral aspects of the eyeball and is continued anteriorly with the transparent cornea. It consists of a firm fibrous membrane that maintains the shape of the eye and gives attachment to the extraocular or extrinsic muscles of the eye.

The cornea is an optically transparent tissue that conveys images to the back of the eye and covers about one-sixth of the total surface areas of the eyeball. It is an avascular tissue to which nutrients and oxygen are supplied *via* bathing with lachrymal fluid and aqueous humour, as well as from blood vessels that line the junction between the cornea and sclera. The cornea is considered to be the main pathway for the permeation of drugs into the eye [13]. It is approximately 0.5 mm thick in the central region, increasing to approximately 0.7 mm at the periphery and is composed of five layers (Fig. 1.2).
The epithelium consists of 5-6 layers of cells, increasing to 8-10 layers at the periphery and has a total thickness of around 50-100 μm and a turnover of about one cell layer per day [2]. The tight junctions and hydrophobic domains in this layer make it the most important barrier against foreign substances including drugs. The basal layer consists of columnar cells, which show extensive lateral interdigitation of plasma membranes and are therefore relatively permeable. The interdigitations may also help to confer mechanical linkage between cells whilst preserving the transparency of the cornea [15]. On top of the basal layer there are two or three layers of polygonal or wing-shaped cells. The uppermost layer consists of non-keratinised squamous superficial cells, which possess microvilli on their anterior surface that may help to anchor the precorneal tear film [16]. Tight junctions (zona occludens) between the superficial cells exclude all solute movement apart from that which occurs by partitioning through the apical and plasma membranes of the surface epithelial cells. The intercellular spaces between the wing cells and between the basal cells are wider than those between the superficial cells. This allows paracellular diffusion of large molecules such as horseradish peroxidase (MW 40,000) [17]. Cell divisions occur in the basal layer of the epithelium and cellular differentiation occurs gradually as cells move
towards the corneal surface. The corneal epithelium is negatively charged at physiological pH [18].

Bowman first described the membrane that bears his name in 1847 [19]. Bowman's membrane is an acellular homogenous sheet consisting of unoriented fibres (8-14 μm) between the basement membrane of the epithelium and the stroma. This membrane is not sharply differentiated from the remainder of the stroma and it may be more accurate to describe it as Bowman's layer; it is likely that it is a specially modified layer of the anterior stroma [19].

The stroma or substantia propria forms around 90% of the corneal thickness. It contains approximately 85% water, and is composed of about 200-250 collagenous lamellae superimposed onto one another and running parallel to the surface. These lamellae consist mainly of regularly arranged collagen fibrils with a uniform diameter of 25-35 nm, with smaller amounts of proteoglycans and corneal fibroblasts (keratocytes) that occupy 2-3% of the total volume of the corneal stroma [20]. These keratocytes repair damage caused by physical or chemical trauma via synthesis and degradation of extracellular matrix material in the stroma in a manner analogous to fibroblasts in the skin [21]. The lamellae lie at right angles to one another in alternating layers and this provides physical strength while permitting optical transparency. The stroma has a relatively open structure and will normally allow the diffusion of solutes with a molecular weight of below 500 kDa. The stroma is easily penetrated by hydrophilic drugs but can act as a barrier against lipophilic drugs that may pass through the epithelium.

Descemet's membrane, which is secreted by the endothelium, is 10-15 μm thick and lies between the stroma and the endothelium.

The corneal endothelium is responsible for maintaining normal corneal hydration and consists of a single layer of flattened hexagonal cells 5 μm high and 20 μm wide. The
endothelium is in direct contact with the anterior chamber and is subject to a passive influx of water from the aqueous humour towards the stroma. The endothelium houses an active Na\(^+/\)K\(^+\) cell pump that prevents swelling of the stroma by regulating corneal hydration and maintaining corneal thickness and thus ensuring corneal transparency. Although tight junctions are present in the endothelium too, it is about 200 times more permeable to water than the epithelium and thus represents a very weak barrier. It has been estimated that drugs with molecular dimensions of about 20 nm are able to diffuse across the normal endothelium [3, 16].

**Choroid**

Very rich in blood vessels and deep chocolate brown in colour, the choroids line the posterior five-sixths of the inner surface of the sclera; when light enters the eye through the pupil, it stimulates the nerve endings in the retina, and is then absorbed by the choroids.

**Ciliary body**

The ciliary body is the anterior continuation of the choroids consisting of ciliary muscles (smooth muscle fibres) and secretory epithelial cells. It gives attachment to the suspensory ligament, which at its other end is attached to the capsule enclosing the lens. Contraction and relaxation of the ciliary muscle changes the thickness of the lens, which bends or refracts light rays entering the eye to focus them on the retina. The epithelial cells secrete aqueous fluid into the anterior segment of the eye. The ciliary body is supplied by parasympathetic branches of the oculomotor nerve (3\(^{rd}\) cranial nerve). Stimulation causes contraction of the smooth muscle and accommodation of the eye.

**Iris**

The iris is the visible coloured part of the eye and extends anteriorly from the ciliary body, lying behind the cornea in front of the lens. It divides the anterior segment of the eye into anterior and posterior chambers that contain aqueous fluid secreted by the ciliary
body. The iris is a circular body composed of pigmented cells and two layers of smooth muscle fibres, one circular and the other radiating. The centre of aperture is known as the pupil. The colour of the iris is genetically determined and depends on the number of pigment cells present. Parasympathetic and sympathetic nerves control the iris: parasympathetic stimulation constricts the pupil and sympathetic stimulation dilates it.

**Lens**

The lens is a highly elastic circular biconvex body lying immediately behind the pupil. It consists of fibres enclosed within a capsule and is suspended from the ciliary body by the suspensory ligament; its thickness is controlled by the ciliary muscle. The lens refracts light rays reflected by objects in front of the eye. The lens is the only structure in the eye that can vary its refractory power, achieved by changing its thickness. When the ciliary muscle contracts, it moves forward releasing its pull on the lens, therefore increasing its thickness. The nearer the object being viewed, the thicker the lens becomes in order to allow focusing.

**Retina**

The retina is the innermost layer of the wall of the eye. It is an extremely delicate structure and is especially adapted for stimulation by light rays. It is composed of several layers of nerve cell bodies and their axons, lying on a pigmented layer of epithelial cells that attach the retina to the choroids. The layer of sensory receptor cells (i.e. rods and cones) is highly sensitive to the light. The retina lines about three quarters of the eyeball and is thickest at the back; it thins out anteriorly to end just behind the ciliary body. Near the centre of its posterior part is the *macula lutea*, or yellow spot. In the centre of this area there is a little depression called the fovea centralis, consisting of cone-shaped cells only. Towards the anterior part of the retina there are fewer cone-shaped than rod-shaped cells. The rods and cones contain photosensitive pigments that convert light rays into nerve
impulses. About 0.5 cm to the nasal side of the macula lutea, all the nerve fibres of the retina converge to form the optic nerve. The small area of retina where the optic nerve leaves the eye is the optic disc, called also *The blind spot* because it has no light sensitive cells.

*Conjunctiva*

This is a fine transparent membrane, which lines the eyelids and the front of the eyeball. The area where it lines the eyelids consists of highly vascular columnar epithelium, while the corneal conjunctiva consists of less vascular stratified epithelium. Conjunctiva plays many roles including protection of ocular surface, production of tear film, and a conduit for drug clearance (depending on drug properties) into the systemic circulation or for drug transport to the deep tissues of the eye [22]. When the eyelids are closed the conjunctiva becomes a closed sac, protecting the delicate cornea and the front of the eye. Usually eye drops are administered by placing them in the lower conjunctival sac. The medial and lateral angles of the eye where the upper and lower lids come together are called the medial canthus and the lateral canthus respectively. The conjunctival epithelium differs somewhat from that of the cornea in that it is thicker and possesses as mucus-secreting goblet cells. The human conjunctiva is between 2 and 30 times more permeable to drugs than the cornea and for this reason it has been proposed that loss by this route is a major path for drug clearance [23].

*The nasolachrymal drainage system*

It consists of three parts, the secretory system, the distributive system and the excretory system. The secretory system consists of basic secretors (lacrimal glands that are stimulated by blinking and temperature change due to tear evaporation) and reflex secretors, which have an efferent parasympathetic nerve supply and secrete in response to physical or emotional stimulation. The lacrimal glands are exocrine glands situated in
recesses in the formal bones on the lateral aspects of each eye just behind the supraorbital margin. Each gland is approximately the size and shape of an almond and is composed of secretory epithelial cells. The glands secrete tears composed of water, mineral salts, antibodies and lysozyme, which is a bactericidal enzyme.

![Diagram of the nasolachrymal drainage system](image)

**Fig. 1.3.** The nasolachrymal drainage system [24].

The distributive system consists of eyelids and the tear meniscus around the lid edges of the open eye that spreads tears over the ocular surface by blinking and thus preventing dry areas from developing.

The excretory part of the nasolachrymal drainage system consists of two lachrymal canaliculi (inferior and superior) and one lachrymal sac and one nasolachrymal duct for each eye. Tears leave the lachrymal gland by several small ducts and pass over the front of the eye under lids towards the medial canthus, where they drain into the two lachrymal canaliculi, the opening of each is called the punctum. The two canaliculi are situated one above the other, separated by a small red body, the caruncle. The tears then drain into the lachrymal sac, which is the upper expanded end of the nasolacrimal duct. This is a membranous canal approximately 2 cm long, extending from the lower part of the lacrimal...
sac to the nasal cavity, which opens at the level of the inferior concha. Normally the rate of secretion of tears keeps pace with the rate of drainage, but when a foreign body or other irritant enters the eye the secretion of tears is greatly increased and the conjunctival blood vessels dilate. Secretion of tears is also increased in emotional states. It is thought that tears are largely adsorbed by the mucous membrane that lines the ducts and the lachrymal sac, and only a small amount reaches the nasal passages [23].

The cul-de-sac of the eye normally holds around 7-9 µl of tears but can retain up to 20-30 µl if care is taken not to blink. The normal tear flow rate is 1 µl / min and the pH is normally maintained between 6.5 and 7.6 [25].

The precorneal tear film is a highly specialised fluid layer that consists of tears and the oily secretion of tarsal glands and covers the corneal epithelium, conjunctiva and the walls of the conjunctival cul-de-sac. The functions of this mixture of fluids include:

- washing away irritating material, i.e. dust, grit,
- preventing microbial infection,
- delaying evaporation and preventing drying of the conjunctiva, and
- nourishment of cornea.

The wandering cells from the limbal vessels reach a central corneal wound via the precorneal tear film and these cells participate in the initial stages of wound healing [26].

Normal secretion of tears by the lachrymal system is also responsible for nutrition of the cornea, the removal of cell debris and foreign substances as well as for protection against bacterial infection.

It was first suggested by Wolff et al. [27] that the tear film has three distinctive layers. The layer adjacent to the corneal epithelium is known as the adsorbed mucin layer, and is composed of glycoprotein. Mucin is secreted by the many goblet cells located on the conjunctival surface, the crypts of Henlé, which are situated on the conjunctival surface of
the upper and lower tarsus, and the glands of Manz, which are positioned in a circular ring on the limbal conjunctiva [24]. Mucin contributes to the stability of the tear film and aids to its attachment of the tear film to the corneal and conjunctival epithelia. The mucin acts as a wetting agent and serves as a bridge between the hydrophobic corneal epithelial surface and the aqueous layer of the tear film that lies immediately above the mucin.

Fig. 1.4. Structure of the tear film [31].

This aqueous layer represents about 98% of the tear film and is composed of water, inorganic salts, glucose and urea as well as biopolymers and various proteins and glycoproteins. This layer has a thickness of around 6-10 μm and is secreted by the accessory lacrimal glands of Kraus and Wolfring, most of which are situated in the upper conjunctival fornix. The upper layer of the tear film is a thin layer of lipid of around 0.1 μm and contains small amounts of mucins and proteins [28]. The lipids, in the form of wax
and cholesterol esters, are secreted from the Meibomian glands and the glands of Zeis situated at the palprebal margin of each eyelid and from the glands of Moll, which are situated at the root of each eyelash [24]. The presence of the lipid layer is believed to retard the evaporation of the middle aqueous layer by 10- and 20-fold, preventing the corneal surface from drying out [29].

Abnormalities of the tear film can lead to dysfunctions of the conjunctiva and eyelids as well as to loss of corneal transparency.

1.3. Ophthalmic disorders

There are several disorders and diseases of the eye that may be treated with the aid of topical ophthalmic formulations. Glaucoma, characterised by an increase in intraocular pressure, has been historically treated with pilocarpine or beta-blockers. Conjunctivitis, which can be caused by a host of environmental, bacterial, viral and fungal agents, may be treated with topical antibiotics, which are commonly formulated in solutions, suspensions or ointments.

Dry eye disorders constitute a considerable proportion of ophthalmological consultations and are the cause of both ocular discomfort and corneal damage in many patients. Such disorders are usually treated with artificial tear preparations, which may contain cellulose derivatives, dextran or polyvinyl alcohol. Iritis is a condition that may occur in association with a number of diseases and disorders and is usually treated with mydriatics and anti-inflammatory drugs.

A wide variety of topical medications are used for treating the numerous diseases and disorders of the eye, Table 1.1. Pilocarpine and timolol are two of the most commonly used anti-glaucoma treatments; due to their need for frequent instillation and long-term
use, such drugs may benefit most from formulation into controlled release systems.

**Glaucoma** represents a group of conditions associated with an increased intraocular pressure due to impaired drainage of the aqueous fluid through the scleral venous sinus (canal of Schlemm) located in the anterior chamber in the angle between the iris and cornea. Persistently raised intraocular pressure may damage the optic nerve by mechanical compression of the axons or by compression of the blood supply, causing ischaemia of the axons. The extent of damage varies from some visual impairment to complete blindness [30].

**Chronic open angle glaucoma.** There is a gradual painless rise in intraocular pressure with progressive loss of vision. Peripheral vision is lost first but may not be noticed until only central vision remains. As the condition progresses, atrophy of the optic disc occurs leading to irreversible blindness. It is commonly bilateral and occurs mostly in people over 40 years of age.

**Acute closed angle glaucoma.** This is most common in people over 40 years of age, and usually affects one eye only. With progressing age, the lenses gradually increase in size pushing the iris forward. In dim light, when the pupil dilates, the lax iris bulges still further forward and may come into contact with the cornea, blocking the scleral venous sinus (canal of Schlemm) causing a sudden rise in the intraocular pressure. Sudden severe pain, photophobia, lacrimation and loss of vision accompany an acute attack. Following repeated attacks spontaneous recovery may be incomplete, leading to progressively impaired vision.

**Chronic closed angle glaucoma.** The intraocular pressure rises gradually without symptoms. Peripheral vision deteriorates, followed by atrophy of the optic disc and blindness.
The function of antiglaucoma medication is to lower the intraocular pressure via one of the following three mechanisms.

i) Increasing the aqueous humour drainage, by producing pupillary constriction (miosis) or directly enhancing drainage through the canal of Schlemm. When the iris is fully constricted, aqueous humour flows easily out of the anterior chamber through the canal. Constriction of the iris is achieved either by directly stimulating cholinergic receptors on the iris or by inhibiting the enzyme that is responsible for terminating the cholinergic transmitter, acetylcholinesterase. Pilocarpine is an example of a direct cholinergic stimulant; phyostigmine and carbachol are anticholinesterases.

ii) Decreasing the amount of aqueous humour production. This may be achieved by using carbonic anhydrase inhibitors that compete for the active site of carbonic anhydrase isoenzyme II, which is found in the epithelium of the ciliary apparatus. This results in decreased production of bicarbonate, and consequently diminished secretion of sodium and bicarbonate into the posterior chamber [32]. This in turn, reduces the amount of aqueous humour formed in the anterior chamber. Topical carbonic anhydrase inhibitors consist of a free sulphonamide group linked to an aromatic ring, for example, acetazolamide. Alternatively, β-blocking agents such as timolol, betaxolol and levobunalol may be used to reduce aqueous humour production. These are either non-selective (work by inhibiting both β₁- and β₂-adrenoceptors) or, preferentially, β₁-selective (inhibit β₁-adrenoceptors).

iii) Reducing intraocular pressure by dehydrating the aqueous humour. This is achieved by making the plasma hypertonic in relation to the aqueous humour, which promotes movement of fluid from the ocular chambers to the plasma. Such
agents include mannitol and glycerine, which induce varying degrees of diuresis [33].

*Conjunctivitis* is an inflammation of the conjunctiva and is a common eye disorder; it may be caused by bacterial and viral infections, or by environmental factors such as pollen, smoke and pollutants. Symptoms include excessive tear production, discharge and pain, but vision remains unaffected.

*Trachoma* is a chronic inflammatory condition, caused by *Chlamydia trachomatis*, in which fibrous tissue forms in the conjunctiva and cornea leading to eyelid deformity: it is a common cause of blindness in tropical countries.

*Blepharitis* is a chronic inflammation of the eyelid margins that is usually caused by microbes or by allergy, e.g. staphylococcal infection or seborrhoea (excessive sebaceous gland secretion). Inflammation caused by a bacterial infection normally responds to antibiotics. Many of the topical ophthalmic antibiotics have a broad spectrum of activity and are used for a number of bacterial infections.

Viral infections of the eye include herpes simplex keratitis, varicella zoster and adenovirus. The general treatment for viral infection requires frequent application of a solution or ointment; instillation on an hourly basis is common. Many of the antiviral agents used interfere with viral DNA synthesis.

*Dry eye syndrome* encompasses a number of separate disease processes, which can lead to an inadequate wetting of the ocular surface. This may be secondary to a deficiency or abnormality of a component of the tear film, or to incomplete spreading of the tear film over the ocular surface. The dry eye syndromes may be divided into five categories: lipid abnormalities, aqueous deficiency (keratoconjunctivitis sicca), mucin deficiency, lid-surfacing abnormalities and epitheliopathy [34]. Inadequate tear production is an important cause of ocular discomfort and often contributes to failure to tolerate contact lenses. The
dry eye condition is common in the elderly. The patient usually complains of a chronic gritty sensation in the eye, which may not appear to be particularly red. Systemic diseases such as rheumatoid arthritis are associated with dry eye. Diuretics may also exacerbate the symptoms of a dry eye. The treatment of dry eye often involves the use of artificial tears, which may be used as necessary. Ointment, which helps to give prolonged lubrication, especially at night when tear secretion is minimal, may be prescribed and acetylcysteine drops may be used if there is clumping of mucus on the eye (filamentary keratitis) [35]. Although artificial tear preparations can relieve the symptoms of dry eye, the major drawback of these treatments is their short duration of action [36].

Keratoconjunctivitis sicca (KCS) is a condition particularly associated with patients suffering from rheumatoid arthritis. Other causes include age-related atrophy of the lachrymal gland, congenital disorders such as Riley-Day syndrome and congenital hypoplasia of the lachrymal gland, trauma and tumours affecting the lachrymal gland, local and systemic inflammation, and neurological and pharmacological effects. Patients suffering from Sjorgen’s syndrome, a chronic systemic disease characterised by polyglandular tissue destruction and involving infiltration of lymphocytes into the lachrymal and salivary glands, develop KCS along with rheumatoid arthritis [37]. The symptoms of keratoconjunctivitis sicca include intermittent burning and tearing, which is exacerbated by reading, drafts, wind, smoke and fumes. Grittiness or foreign body sensation, itching and redness are also common. The precomeal tear film may contain a considerable amount of cellular debris, which is inadequately flushed from the ocular surface. Abnormal accumulation of mucus also occurs, leading to the formation of prominent strands, which tend to settle in the inferior cul-de-sac. This is thought, again, to be the result of poor flushing action by the aqueous layer and is usually treated with artificial tears, which are instilled as required by the patient. Other treatments have been
used in conjunction with tear replacement therapy, including inhibition of tear drainage, stimulation of tear production, bandage contact lenses and the use of anti-inflammatory agents and mucolytic agents [34].

*Keratitis* may be caused by a variety of agents and often leads to ulceration of the cornea. A corneal ulcer is defined as a breach of the corneal epithelium other than a traumatic abrasion. Causes of corneal ulceration include bacterial, viral and fungal infection (such as with *Candida albicans*), the presence of a foreign body and toxic reactions. Contact lenses represent the first suspect in cases of keratitis. The herpes simplex virus is also a common cause of keratitis; this may affect any of the corneal tissues, and can lead to epithelial keratitis, disciform keratitis, stromal keratitis and iritis. Treatment for such infections may take a variety of forms including topical trifluridine or orally administered acyclovir for epithelial keratitis; topical corticosteroids combined with an antiviral are used in cases of disciform and stromal keratitis and iritis [38]. Other forms of keratitis include neuroparalytic keratitis and *Rosacea keratitis*, which is a chronic disorder of the face involving reddening and inflammation of the skin. Ophthalmic complications associated with this condition include inflammation of the lid margins (blepharitis), meibomian cysts of the eyelid (chalazia), conjunctivitis and keratitis. Treatment is usually with low dosage oral tetracycline [25]. Neuroparalytic keratitis arises when there is loss of corneal sensation, which may result from a herpes zoster infection, or any lesion of the fifth nerve. This condition may be treated with the copious use of ointment at night or with ‘bandage’ soft contact lenses.

*Iritis* also referred to as anterior uveitis, is a condition that may occur in association with diseases such as ankylosing spondylitis, sarcoidosis, tuberculosis and syphilis; it is commonly seen in patients following intraocular surgery, ocular trauma and in those suffering from corneal ulcers. Commonly, iritis has an acute onset, with the patient
suffering with pain and inflammation of the eye. Pain may be quite severe and is partly due to the spasm of the ciliary muscle. The vision is often blurred and there may be some photophobia and watering of the eye. It is not uncommon for the second eye to become infected a few days later. The inflamed iris becomes sticky with the pupil margin often becoming adhered to the lens. Initially, the points of adhesions are difficult to detect but these become apparent upon instillation of a mydriatic agent as the pupil dilates irregularly. If the entire pupillary margin is adhered to the lens, the aqueous humour is prevented from passing from the posterior to the anterior chamber. Aqueous humour accumulates rapidly behind the iris and pushes it forward; a rise in intraocular pressure follows and can lead to secondary glaucoma. The treatment of iritis is directed towards the control of inflammation, the breakdown of adhesive interactions and the relief of pain. Anti-inflammatory drugs are used in conjunction with mydriatics to dilate the pupil, thus separating the iris from the lens. Pain usually subsides upon successful treatment of the other symptoms but analgesics may also be prescribed [14].

1.4. Commonly used ophthalmic drugs

A wide variety of topical ophthalmic medications are used for treating the numerous diseases and disorders of the eye; these are summarised in Table 1.1. Pilocarpine and timolol are just two of the most commonly used anti-glaucoma treatments. Antibiotics, e.g. chloramphenicol and norfloxacin, are also common in use for treating various kinds of bacterial infections.
Table 1.1. Ophthalmic therapeutic agents [33].

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical anaesthetics</td>
<td>Cocaine</td>
</tr>
<tr>
<td></td>
<td>Proparacaine</td>
</tr>
<tr>
<td></td>
<td>Tetracaine</td>
</tr>
<tr>
<td></td>
<td>Benoxinate hydrochloride</td>
</tr>
<tr>
<td></td>
<td>Dibucaine hydrochloride</td>
</tr>
<tr>
<td></td>
<td>Ketorolac</td>
</tr>
</tbody>
</table>
| Topical mydriatics and combined mydriatics and cycloplegics | Phenylephrine
|                                | Atropine                                       |
|                                | Homatropine hydrobromide                       |
|                                | Cyclopentolate hydrochloride                   |
|                                | Scopolamine hydrobromide                       |
|                                | Tropicamide                                    |
|                                | Dipirefrin hydrochloride                       |
| Vasoconstrictor                | Phenylephrine hydrochloride                    |
| Topical antibiotics            | Bacitracin                                      |
|                                | Chloramphenicol                                |
|                                | Aminoglycosides (neomycin, gentamycin, tobramycin) |
|                                | Penicillins and cephalosporins                 |
|                                | Sulphonamides                                  |
|                                | Tetracycline                                   |
| Antiglaucoma medications       | Muscarinic agonist (Pilocarpine, Carbachol)    |
|                                | Anticholinesterase agents (physostigmine, neostigmine, isofluorophate, echothiophate) |
|                                | Sympathomimetics (epinephrine)                 |
|                                | Beta-adrenergic–blocking agents (timolol, betaxolol, levobunalol) |
|                                | Carbonic anhydrase inhibitors (acetazolamide, dorazolamide) |
|                                | Hyperosmotic agents (mannitol, glycerine, isosorbide) |
|                                | Alpha-2 adrenoceptor agonist (clonidine)       |
|                                | Prostaglandin analogue (latanoprost)           |
| Topical antiviral agents       | Acyclovir, Gancyclovir                          |
|                                | Idoxuridine                                    |
|                                | Viadarabine                                    |
|                                | Trifluridine                                   |
| Topical antifungal agents      | Natamycin                                      |
|                                | Nystatin                                       |
| Topical corticosteroids        | Prednisolone                                   |
|                                | Dexamethasone                                  |
|                                | Betamethasone                                  |
Pilocarpine

Pilocarpine, a derivative of the plant genus *Pilocarpus*, is one of several alkaloids that mimic the effect of acetylcholine on the autonomic effector cells that control smooth muscle contraction. It has been used in the treatment of glaucoma for more than a century. The compound, (Fig. 1.5) binds to the muscarinic class of acetylcholine receptors and exhibits partial agonist activity [39].

![Fig. 1.5. Structure of Pilocarpine](image)

Pilocarpine solutions ranging from 0.5 to 10 % have been used in the treatment of glaucoma. The usual therapeutic concentration is 2 %, which is administered as one to two 50 µl drops for up to 4 times per day. Pilocarpine is also available as a gel preparation containing 4 % drug, which is applied once per day. The Ocusert® Pilo 40 system offers an alternative to eyedrops and gels and takes the form of an insert, which, when placed into the upper fornix, is able to release pilocarpine for up to one week at an average rate of 40 micrograms per hour [40]. Pilocarpine reduces intraocular pressure by about 20 to 30 % [41].

Although pilocarpine is considered to be the safest and most effective form of anti-glaucoma medication, it has several characteristics and side effects that can limit its use. It has a short duration of action (4 to 6 hours) and aqueous drops must be administered four times per day. Miosis decreases the amount of light that enters the eye and thus dims the sense of vision and prolongs dark adaptation. In younger patients, the contraction of the ciliary muscle by pilocarpine induces artificial shortsightedness and can also cause
uncomfortable muscle spasms. Other adverse effects include conjunctival hyperaemia, lens opacities and retinal detachment. Systemic effects are also observed when higher concentrations of pilocarpine are used; these include vomiting, nausea, diarrhoea, tachycardia, bronchospasm and sweating [41]. In aqueous solution, pilocarpine presents at least two possible pathways of degradation: hydrolysis to pilocarpic acid and epimerisation to isopilocarpine. Pilocarpine is relatively stable in acidic solutions but as the pH is increased, it progressively becomes unstable, especially at elevated temperatures. The addition of 0.5% methylcellulose has been shown to improve the stability of pilocarpine solutions [42].

**Timolol**

Timolol is a non-selective, β-adrenergic blocking agent that reduces intraocular pressure by inhibiting aqueous humour production. The precise mode of action of timolol is unclear, but it has been suggested that timolol down-regulates the adenylate cyclase enzyme by inhibiting β2-adrenoceptor sites at the ciliary processes. Clinical studies have reported 27-35% reductions of intraocular pressure during long-term treatment [41]. Timolol is available in 0.1, 0.25 and 0.5% solutions and is normally applied twice daily. A formulation employing a gel as a vehicle (Timoptic-XE®) permits once-daily application. Timolol causes fewer ocular adverse effects than miotic or sympathomimetic drugs but its use reduces tear flow and can result in dry eye syndrome or give difficulties for contact lens wearers. However, β-blockers may induce adverse systemic effects by blocking the β1-adrenoceptors of the heart. Timolol may cause bradycardia, arrhythmia and congestive heart failure. In addition, it can cause bronchospasm in patients with chronic obstructive pulmonary disease and asthma. Furthermore, timolol may induce anxiety, depression, fatigue, disorientation and hallucinations. All these systemic side effects are caused by the immediate uptake of timolol in the blood via the epithelium of the nasopharynx. Plasma
levels of timolol after instillation can be reduced by up to 70% by compression of the nasolachrymal punctum at the time of application, thereby minimising the unwanted systemic effects [41].

Antibiotics

Examples include bacitracin, commercially available as an ointment, and chloramphenicol, manufactured as both a solution and an ointment. Bacitracin is a broad-coverage antibiotic that inhibits bacterial cell wall synthesis and is effective against gram-positive cocci, bacilli, Neisseria, Haemophilus and spirochaetes. Chloramphenicol inhibits bacterial protein synthesis by competing with binding of the 50S ribosomal unit. This compound is active against gram-positive, gram-negative and anaerobic organisms and achieves good penetration into the corneal epithelium. Acyclovir is the most commonly prescribed antiviral, although idoxuridine has been used against herpes simplex keratitis and works by replacing viral thymidine [43]. Vidarabine is effective against herpes simplex keratitis, varicella-zostoe; however, its administration may delay corneal regeneration. Trifluridine is used against herpes simplex and adenovirus infections. Side effects may include swelling of the eyelids, photophobia, stinging upon administration and increased intraocular pressure [44].

Norfloxacin

Norfloxacin is an oral broad-spectrum quinoline antibacterial agent used in the treatment of various ophthalmic disorders. The mechanism of action of norfloxacin involves inhibition of the A subunit of the important bacterial enzyme DNA gyrase, which
is essential for DNA replication [45]. The most common adverse effects are gastrointestinal, neuropsychiatric and skin reactions, and include nausea, headache, dizziness, rash, heartburn, abdominal pain/cramps and diarrhoea. Frequent applications of topical norfloxacin in patients with decreased tear secretion may result in deposition of drug onto the cornea [46].

Atropine

Atropine is a tropane alkloid extracted from the deadly nightshade (Atropa belladonna) and other plants of the family Solanaceae. It is a secondary metabolite of these plants and serves as a drug with a wide variety of effects.

![Fig. 1.7. Structure of Atropine](image)

Atropine binds to the muscarinic class of acetylcholine receptors and exhibits antagonist activity. Topical atropine is used as a cycloplegic, to temporarily paralyze accommodation, and as a mydriatic, to dilate the pupils. It degrades slowly, typically wearing off in 2 to 3 days, so tropicamide is generally preferred as a mydriatic. In atropine-induced mydriasis, the mechanism of action involves blocking the contraction of the circular pupillary sphincter muscle (which is normally stimulated by acetylcholine release), thereby allowing the radial pupillary dilator muscle to contract and dilate the pupil. It is contraindicated in patients predisposed to narrow angle glaucoma. Side effects may include urinary retention, dry mouth and blurred vision. The active is a racemic mixture of D-hyoscyamine and L-hyoscyamine, with most of its physiological effects due to L-
hyoscyamine. The most common atropine compound used in medicine is atropine sulfate hydrate: \( \text{C}_{17}\text{H}_{23}\text{N}_{3}\text{O}_{3} \cdot \text{H}_{2}\text{SO}_{4} \cdot \text{H}_{2}\text{O} \). \( 1\alpha\)-H, \( 5\alpha\)-H-tropan-3-\( \alpha \)-ol (±)-tropate(ester), sulfate monohydrate.

1.5. Drug delivery to the eye

The complicated removal mechanisms in the precorneal area (Section 1.1) are necessary for the protection of the eye from foreign substances. In order to design a drug delivery vehicle that could safely overcome these mechanisms, it is necessary to appreciate the disposition pathways of the drug molecule and its ocular pharmacokinetic/pharmacodynamic profile. The various types of ophthalmic drug delivery carriers are also described in this section.

1.5.1. Ocular pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of the absorption, distribution and elimination of a drug and describes the quantitative relationship between the administered dose, the dosing regimen and the observed plasma or tissue concentration. Pharmacodynamics may be described as the quantitative relationship between observed plasma or tissue concentration of the active form of the drug and the corresponding pharmacological effect. Pharmacokinetic analysis of drugs is often approached by dividing the body, or organ of interest, into a series of compartments, which mathematically represent time variations of levels of drug in tissue as a summation of exponentials.

Ocular pharmacokinetic studies are generally undertaken using the rabbit eye as the model organ. It is not possible to carry out ocular pharmacokinetic studies in humans.
without risking pain or injury. Although the rabbit eye is useful for predicting human ocular toxicities, the eyes of each species have a number of differences in anatomy and physiology, such that predicting human ocular pharmacokinetics from rabbit data may not be very precise for certain drugs (Table 1.2).

Table 1.2. Comparative anatomical and physiological features of New Zealand rabbit eye vs human eye

<table>
<thead>
<tr>
<th>Pharmacokinetic Factor</th>
<th>Rabbit Eye</th>
<th>Human Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tear volume</td>
<td>7.5 μl</td>
<td>7.0-30.0 μl</td>
</tr>
<tr>
<td>Tear turnover rate</td>
<td>0.6-0.8 μl/min</td>
<td>0.5-2.2 μl/min</td>
</tr>
<tr>
<td>Spontaneous blink rate</td>
<td>4-5 / min</td>
<td>15 / min</td>
</tr>
<tr>
<td>Nictitating membrane</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>pH of tears</td>
<td>7.14-7.82</td>
<td>7.14-7.82</td>
</tr>
<tr>
<td>Milliosmolarity of tears</td>
<td>305 mOsm/l</td>
<td>305 mOsm/l</td>
</tr>
<tr>
<td>Corneal thickness</td>
<td>0.40 mm</td>
<td>0.52 mm</td>
</tr>
<tr>
<td>Corneal diameter</td>
<td>15 mm</td>
<td>12 mm</td>
</tr>
<tr>
<td>Aqueous humour volume</td>
<td>310 μl</td>
<td>310 μl</td>
</tr>
<tr>
<td>Aqueous humour turnover rate</td>
<td>1.53 μl/min</td>
<td>1.53 μl/min</td>
</tr>
</tbody>
</table>

The single compartment model (Fig. 1.8) represents the simplest pharmacokinetic model for the eye.

![Fig. 1.8. Schematic of one-compartment model](image)

The expression that is used to describe this process in terms of drug concentration, \( C \), is:
\[ C = \left( \frac{FD}{V_d} \right) \left( \frac{k}{k-K} \right) \left( e^{-k' \tau} - e^{-k \tau} \right) \]

where \( D \) is the dose, \( F \) is the fraction of dose absorbed, \( k \) and \( K \) are the absorption and elimination rate constants, respectively, and \( V_d \) is the apparent volume of distribution.

However, it may be more appropriate to consider the various tissues of the eye as separate compartments. Lee and Robinson [47] considered some of the pathways accounting for drug loss in the precorneal area by means of a four-compartment model (Fig. 1.9) capable of predicting precorneal and intraocular drug movement following topical dosing.

![Diagram](image)

**Fig. 1.9. Model depicting precorneal and intraocular drug movement [47]**

Drug penetration through the cornea and lens may be analysed in terms of a diffusion process. The iris-ciliary body and lens may be considered as reservoirs that bind drugs and release them slowly. Makoid and Robinson [48] considered a four-compartment model - consisting of the precorneal area, cornea, aqueous humour and reservoir tissues
(lens, iris etc.) – in which there is a two-way movement of drug between cornea and aqueous humour and between aqueous humour and the reservoir tissue. Excellent correlation between predicted and observed values was seen in multiple dosing studies. Furthermore, it was found that the drainage rate of pilocarpine at the precorneal area was one to two orders of magnitude greater than the rate of corneal absorption.

1.5.2. Ophthalmic drug delivery vehicles

Although there are a wide variety of ophthalmic drug delivery systems on the market, 70% of prescriptions for eye medication are for conventional eye drops. This is due to a number of issues related to the other types of vehicle that have been developed and tested over the past 20-30 years, namely: cost, difficulty in bulk manufacture, patient compliance, efficacy and stability. The various types of ocular drug delivery vehicles in existence are discussed in the following sections.

1.5.2.1. Eye drop solutions and suspensions

Eyedrops are the most commonly used of all dosage forms and account for around 90% of currently available ophthalmic formulations [4], because they can be administered in the form of solutions or suspensions and are convenient, safe, immediately active and, with the exception of the very young and the elderly, are well accepted by patients. However, these dosage forms require frequent instillations of highly concentrated solutions due to the rapid and extensive precorneal loss caused by nasolacrimal drainage and high tear turnover. Typically less than 5% of instilled drug penetrates the cornea and reaches intraocular tissues, while a significant fraction of the dose is absorbed systemically [49,50]. Eye drops facilitate a pulse-entry of the drug, which is followed by a rapid decline in the
drug concentration in the tears, the kinetics of this process being of approximate first order [24].

The two major physical forms of eyedrops are aqueous solutions and suspensions. Most of the major ophthalmic therapeutic agents are water-soluble or can be formulated as water-soluble salts. The most common salt forms are the hydrochloride, sulphate, nitrate and phosphate; the selection of the appropriate salt being determined by solubility, therapeutic concentration, effect of pH, tonicity and buffer capacity. The adjustment of the pH of ophthalmic solutions is important as this can have implications for comfort, activity and stability of the product. The ideal pH is considered to be 7.4, as this is the value of normal physiological tear fluid and thus less likely to promote increased tear production, which, in turn, may result in excessive loss of drug. Nonetheless, in vivo experiments in rabbits have shown that an increased pH can enhance drug permeability across the cornea, owing to the solution containing a higher concentration of the non-ionised drug base [51]. Suhonen et al. [52] found that an increase in the pH of pilocarpine preparations from 5.5 to 7.65 brings a three-fold increase in permeability across the isolated rabbit cornea. However, in order to achieve adequate stability and solubility for an acceptable shelf-life of at least two years, pilocarpine and its prodrugs must be formulated at an acidic pH solution, being neutralised following instillation on the ocular surface [53].

Ophthalmic solutions and suspensions should be sterile when dispensed, and a suitable preservative must be added to this. Eyedrop preparations are usually sterilised by autoclaving, although in some cases, due to their lack of thermostability at physiological pH, bacterial filtration may be used as an alternative method e.g. for basic salts of weak acids such as sodium fluorescein. Suitable preservatives for eyedrops include benzalkonium chloride, chlorobutanol, phenylmercuric acetate and thiomersal. These are topically non-irritating antibacterial agents that prevent growth of, or destroy,
microorganisms that may be accidentally introduced into the eyedrop container during use. Ophthalmic solutions and suspensions are usually packaged in glass or plastic containers incorporating a dropper. Such types of packaging are subject to contamination during use, which may be airborne or be brought about by the touching of the tip of the dropper on the ocular tissues. Eyedrops in multiple-application containers for domiciliary use should not be used for more than four weeks after first opening (unless otherwise stated). Solutions and suspensions for use in hospital wards are normally discarded one week after first opening; individual containers are provided for each patient. Eyedrops used in outpatient departments are usually discarded at the end of each day (British National Formulary, 2001). The prescribed amount of ophthalmic solution or suspension is dropped quickly, but completely, into the eye without touching the dropper to the eye or surrounding tissue. If the patient is administering the preparation unaided, the dropper should be held above the eye with one hand, while the other is used to pull down the lower eyelid. The medicine is then dropped into the eye, while the patient looks upward. The patient should remain in place with the eye open for 30 seconds and should be instructed not to close the eyes tightly or blink more than is usual [53]. These recommendations for the correct administration of ophthalmic solutions and suspensions are given in order to obtain the maximum therapeutic effect from the prescribed dose. However, many patients, particularly the elderly and the very young, find this procedure difficult to coordinate and thus fail to administer the correct dose.

1.5.2.2. *Viscous vehicles*

Polymers are frequently added to ophthalmic solutions and suspensions in order to increase the ocular bioavailability of drugs by increasing the viscosity of the vehicle [54], thereby prolonging contact with the cornea. Such polymers are typically high molecular weight hydrophilic molecules (5000-100000 Da) that are unlikely to cross biological
membranes, and include synthetic polymers such as: polyvinyl alcohol; poly(vinylpyrrolidone); polyacrylic acid and poloxamers; animal polysaccharides (such as hyaluronic acid); or cellulose derivatives (methylcellulose, hydroxymethylcellulose and carboxymethylcellulose) [56,58]. Other polymers include microbial polysaccharides such as dextran, xanthan and gellan. Gellan is available as Gelrite®, a commercial preparation, which is obtained by the acetylation of native gellan [55]. Beside medicated eyedrops, viscous solutions for ophthalmic preparations are also used for artificial tears and contact lens solutions.

The useful viscosity range of an ophthalmic solution often represents a limiting factor in the use of viscous vehicles. It has been reported that an increase in the corneal penetration of an ophthalmic drug is maximal at a viscosity of about 15 to 150 cP [57]. Any further viscosity increase is reported to have little effect on the drainage rate and tear film thickness and may be associated with interference with vision and resistance to the movement of the eyelids [57].

Low viscosity Newtonian polymer solutions applied to the precorneal area effect little improvement in bioavailability, whereas at high viscosity such solutions do not increase residence contact time whilst making blinking painful [59]. Non-Newtonian formulations - that display pseudoplastic properties in which the viscosity decreases with increasing shear rate - offer significantly less resistance to blinking. Such formulations are accepted by patients more readily than viscous, newtonian formulations [16].

Chrai and Robinson [60] showed that the rate of drainage of a viscous methylcellulose solution containing pilocarpine decreased with increasing viscosity of the solution. They found a linear first order relationship between the drainage rate and both the miotic activity and drug concentration in the aqueous humour. Poly(vinylalcohol) is a synthetic, long chain polymer that has been used in ophthalmic preparations since the early
1960s. Poly(vinylalcohol) can lower the surface tension of water and enhance tear film stability. These properties have led to the widespread use of poly(vinylalcohol) as a drug delivery vehicle and artificial tear preparation. It has been shown that when poly(vinylalcohol) and methylcellulose are compared on a viscosity basis, there is no difference between the two vehicles in terms of their influence on ocular drug bioavailability in rabbit [57]. Meseuger et al. [61] found that even after 20 minutes, viscous solutions of xanthan gum and Gelrite®, containing a radioactive label ($^{99m}$Technetium), were able to facilitate a level of radioactivity in the human eye that was at least equal to the level achieved with a non-viscous reference solution after 1 min. Poly(vinylpyrrolidone), PVP, has also been employed as a viscosity-enhancing agent in ophthalmic solutions. Nagarsenker et al [62] found that isoviscous solutions of PVP, poly(vinylalcohol), hyaluronic acid and hydroxypropylcellulose all reduced the systemic absorption of timolol, but to varying extents; PVP was found to be the most effective in enhancing timolol absorption.

Polymers that enhance the viscosity of eyedrop preparations may be subdivided into: non-mucoadhesive polymers, bioadhesive-mucoadhesive polymers, *in situ* gelling systems and colloidal systems (liposomes, micro-particles and nanoparticles); these will be discussed in more detail in later sections.

1.5.2.3. Colloidal systems

Colloidal systems, encompassing liposomes as well as micro- and nano-particles, have been studied widely over the past 20 years as a means of increasing specificity of drug action, enhancing bioavailability, and protecting the active against enzyme inactivation [4]. Colloidal particles are subjected to the same clearance mechanisms as other foreign bodies that may come into contact with the ocular surface and tend to be
washed away by reflex tearing. Particles of larger size are more likely to be entrapped under the eyelids or in the inner canthus and so remain in contact with the corneal and conjunctival epithelia for extended periods. However, large particles in the eye are uncomfortable for the patient; it is generally accepted that particles intended for ophthalmic use should not exceed 5-10 μm in diameter.

Liposomes are membrane-like vesicles, consisting of lipid bi-layers that surround an aqueous compartment; the lipid layers are composed mainly of phospholipids. Liposomes are classified according to their size and are known as either small unilamellar vesicles (10-100 nm), large unilamellar vesicles (100-3000 nm) or, if a number of bi-layers are present, multilamellar vesicles. The charge on a liposome may be positive, negative or neutral depending on its components. If the external membrane is composed of stearylamine, the liposome carries a positive charge, if it is made of dicetylphosphate or phosphatidic acid, the liposome carries a negative charge and if the outer layer is phosphatidylcholine or dipalmitoylphosphatidylcholine, the liposome is neutral. Fitzgerald et al. [11] used γ-scintigraphy to evaluate the precorneal drainage of 99mTc labelled liposomes in rabbits. The liposomes were shown to have prolonged association with the ocular tissues but were found to be poorly retained on the corneal surface. However, more recent studies have confirmed that certain positively charged liposomes are capable of increasing precorneal retention and enhancing drug bioavailability [11, 63]. This behaviour has been attributed to interactions with the negatively charged corneal surface, which has an electrical potential of -30 mV [16]. The use of polymer-coated vesicles as a means of prolonging contact between liposomes and the corneal surface has also been investigated. Multilamellar vesicles coated with the mucoadhesive polymer Carbopol have been prepared by Davies et al. [64], who have reported that at pH 5 the polymer-coated liposomes were retained in the eye for a significantly longer period than
uncoated vesicles. Durrani et al. [31] found that although the *in vivo* use of coated liposomes containing pilocarpine imparted a larger area under the miotic intensity-time curve and a longer duration of action as compared with uncoated liposomes, showed equal activity to a pilocarpine reference solution. In addition, the potential of liposomes as a topical ophthalmic drug delivery system appears to be limited by their drug-loading capability and their stability. Furthermore, the large-scale manufacture of liposomes is expensive and technically challenging.

Nanoparticles are solid colloidal drug carriers ranging in diameter from $10$ to $1000$ nm and the drug may be entrapped within the particle or adsorbed onto its surface. Nanoparticles prepared for ophthalmic drug delivery have mainly been produced by emulsion polymerisation of synthetic monomers; particle size is controlled by the size of the emulsion [65,67].

Microparticles are microcapsules or microspheres that have a particle diameter exceeding $1 \, \mu m$. Microspheres are monolithic particles possessing a porous or solid polymer matrix, whereas microcapsules consist of a polymeric membrane surrounding a solid or liquid drug reservoir [69]. Upon topical instillation, the particles tend to reside in the ocular cul-de-sac, and the drug is released from the particles through diffusion or as a result of polymer degradation [10].

A large number of methods are available for the production of micro- and nanoparticles, with the method of choice being determined by the drug to be used [71]. Techniques for the manufacture of particles include denaturation or crosslinking of macromolecular emulsions [67, 68], interfacial polymerisation [70, 72], formation of emulsions followed by solvent removal [61], solution enhanced dispersion by supercritical fluids [74] and spray drying [75, 76]. A wide range of polymers has been used in the manufacture of micro- and nanoparticles for ophthalmic drug delivery. These include
poly(alkyl)cyanoacrylate, used to encapsulate drugs such as timolol; polylactic acid for the delivery of chloramphenicol, cyclosporine A and pilocarpine; and albumin, which can be formulated to deliver hydrocortisone and pilocarpine [73,77]. In an early study, Gurney et al. [78] developed a cellulose acetate-hydrogen phthalate pseudolatex formulation for the delivery of pilocarpine. The same workers found that following administration of this formulation both the miosis $t_{max}$ (time to reach the maximum miotic effect) and the area under the miotic intensity-time curve were increased by 50 %, compared to an aqueous reference solution. However, this has been disputed by Zimmer et al., who claimed that the observed pharmacological enhancement was due to an increase in viscosity of the vehicle, and was not caused by a slow release of drug from the particles [73]. Giunchedi et al. [75] found that the bioavailability of piroxicam, a non-steroidal anti-inflammatory drug (NSAID) used for the topical treatment of ocular inflammation, could be increased three-fold if formulated as pectin microspheres (prepared by spray drying), as compared with a commercial reference solution. Other studies have shown that the uses of micro- and nanoparticles in ocular drug delivery are capable of slowing the release profile of pilocarpine, and as a result prolong pharmacological responses (such as miosis) and reduced intraocular pressure [79]. Recent studies have shown that the use of mucoadhesive polymeric nanoparticles is a promising means of achieving selective and prolonged drug delivery to the eye [31]. Kao et al. [80] found that pilocarpine/chitosan/carbopol nanoparticles may provide an excellent potential alternative ophthalmic sustained-release formulation of pilocarpine for clinical use. Zimmer et al. [73] investigated the influence of a number of viscous and bioadhesive polymers (methylcellulose, poly(vinylalcohol), hydroxypropylmethylcellulose, hyaluronic acid, mucin, sodium carboxymethylcellulose, polyacrylic acid) on the $in vivo$ activity of pilocarpine-loaded albumin nanoparticles. Bioadhesive polymers are reported to have superior effects as compared to their viscous
counterparts, with the area under the miotic curve being seen to increase 1.35-fold following the addition of polyacrylic acid, and 1.64-fold when mucin was employed. One of the approaches recently developed involves drug incorporation into cationic submicronic polymeric vectors (such as chitosan), which exploit the negative charges present at the corneal surface for increased residence time and penetration [82].

1.5.2.4. Ointments and gels/hydrogels

Ointments are semisolid preparations intended for external application. Ointments may be medicated or nonmedicated, the latter type being commonly referred to as ointment bases and used as such for their emollient or lubricating effect, or utilised as vehicles in the preparation of medicated ointments. Ophthalmic ointments are usually formulated using petrolatum, a mixture of semisolid hydrocarbons obtained from petroleum, and liquid petrolatum (mineral oil). These bases have a melting or softening point close to body temperature and should be non-irritating to the eye. Ointments are characterised as simple bases, where the ointment forms one continuous phase, and compound bases in which an emulsion of oil and water is formed. The medicinal agent is added to the base either as a solution or as a finely micronised powder. Upon instillation in the eye, ointments break up into small droplets and may remain as a depot of drug in the cul-de-sac for extended periods. Ointments are therefore useful for improving drug bioavailability and for sustaining drug release into the ocular tissues. The major use of ointments is in the delivery of antibiotics, sulphonamides, antifungals and anti-inflammatories.

Sieg and Robinson [81] have demonstrated that ointments exhibit extended effect of steroidal drug fluorometholone in aqueous humour levels and enhance the percentage of drug penetrating the ocular tissues, as compared with suspensions of the same active. The same workers reported that partitioning of the steroid from the ointment had a greater rate-
limiting effect on corneal penetration than the dissolution rate parameter characterising the aqueous suspension. Although ointments are safe, they suffer with relatively poor patient compliance due to refractive index problems and occasional ocular mucosal irritation [82, 83]. For these reasons they are often used as a night-time medication.

An alternative to ointments is the use of aqueous gels, commonly known as hydrogels. These consist of high molecular weight, hydrophilic crosslinked polymers or co-polymers that form a three-dimensional network, which can hold water or an aqueous solution containing a drug [85]. A gel of this type usually consists of macromolecules existing as twisted matted strands where the units are often bound together by van der Waals’ forces. Hydrogels have been shown to bestow significantly longer residence times in the conjunctival cul-de-sac and increased drug bioavailability, thereby prolonging the therapeutic level in the eye [86,87,89]. Oxygen transmission requirements have been addressed through the use of siloxane and fluorosiloxane containing hydrogels [91]. Hydrogels may be prepared by polymerising water-soluble monomers in the presence of bi- or multifunctional crosslinking agents. Alternatively, chemical gels can be prepared by crosslinking water-soluble polymers, by using functional group chemistry. However, the crosslinking agents used are often toxic compounds, which have to be extracted from the gels before they can be applied. Moreover, crosslinking agents can give unwanted reactions with the bioactive substances present in the hydrogel matrix. Such adverse effects are avoided with the use of physically crosslinked gels [93].

Hydrogels may be classified into two groups: pre-formed and in situ forming gels. Pre-formed gels may be defined simply as highly viscous solutions whereas in situ gelling systems are usually viscous liquids that shift to a gel phase upon exposure to physiological conditions [95]. Pre-formed gels include the cellulose derivatives, polyvinyl alcohol, hyaluronic acid and carbomer.
1.5.2.5. Solid matrices and devices

A number of solid polymeric inserts and discs have been developed as ophthalmic drug delivery systems. In contrast to eyedrops, inserts provide accurate dosing, reduced systemic absorption and, in some cases, improve patient compliance (resulting from a reduced frequency of administration and lower incidence of visual and systemic side-effects) [97,99]. The performance of inserts is little affected by nasolachrymal drainage and tear flow, with such devices having been shown to produce reliable drug release over prolonged residence times in the conjunctival cul-de-sac [83].

Ocular inserts have been investigated for the purpose of delivering a range of therapeutic agents including antimicrobials, antivirals, antifungals, antiallergenics, anti-inflammatories (steroidal and non-steroidal), antiglaucoma medications, immunosuppressants and growth factors [86]. A number of inserts are currently available on the market or are in the latter stages of development by pharmaceutical corporations, including Ocusert®, Soluble Ocular Drug Insert (S.O.D.I), Collagen shields, Ocufit®, Minidisc and the Novel Ophthalmic Delivery System (NODS®). Several workers have investigated Gelfoam® - an absorbable gelatine sponge prepared from purified porcine skin and usually used for application to areas of bleeding as a haemostatic - as the basis of a topical ophthalmic insert [88,90,92,94]. Inserts have been classified, on the basis of their physico-chemical behaviour, as degradable or non-degradable. Degradable inserts are monolithic polymeric devices that undergo gradual dissolution, or erosion, while releasing an incorporated drug; these do not require removal. Various materials have been utilised in the development of degradable inserts, including polyvinyl alcohol, hydroxypropylcellulose, N-vinylpyrrolidone, poly(vinyl methyl ether/ maleic anhydride), xanthan gum, poly(lactic acid) and poly(lactic-glycolic acid) and hyaluronic acid [101]. Commercial examples include Soluble Ocular Drug Inserts (S.O.D.I), collagen shields and
Novel Ophthalmic Delivery Systems (NODS®). Non-degradable inserts must be removed from the eye after use and have been shown to provide more predictable release rates than their soluble counterparts [83]. Such devices have been prepared from ethylene/vinyl alcohol copolymers, ethylene/vinyl acetate copolymers, styrene-isoprene-styrene block copolymer, poly(lactic acid-co-glycolic acid) and poly(2-hydroxypropyl methacrylate). Hydrogels have also been investigated as non-degradable matrices and they have been found to provide a constant drug release rate conforming to a non-Fickian diffusion mechanism. Commercial examples of non-degradable inserts include Ocusert®, Ocufit® and Minidisc. The Soluble Ocular Drug Insert (S.O.D.I.) is a small oval wafer, which was developed by Soviet scientists for cosmonauts who were unable to use eyedrops in weightless conditions; this was first described in 1976 by Maichuk [96]. The Soluble Ocular Drug Insert is composed of a soluble copolymer, consisting of acrylamide, N-vinylpyrrolidone and ethyl acrylate. The inserts are thin, homogenous polymer platelets with dimensions 9 mm x 4.5 mm, a thickness of 0.35 mm and weighing 15-16 mg. After introduction into the upper conjunctival sac, the Soluble Ocular Drug Insert softens within 10-15 seconds, conforming to the shape of the eyeball. The insert forms a polymer clot over the next 10-15 minutes and gradually dissolves within one hour, while releasing an incorporated drug. Over 20 common ophthalmic drugs and drug combinations have been delivered using the S.O.D.I. Maichuk and Erichev [98] showed that the daily administration of a S.O.D.I containing 2.7 mg pilocarpine hydrochloride effectively reduced the intraocular pressure in 155 glaucoma patients.

The Novel Ophthalmic Delivery System (N.O.D.S.) is a water-soluble film patented by Smith and Nephew Pharmaceuticals Ltd. in 1985. It was developed in an attempt to give precise and controlled drug delivery to the eye. The device consists of a medicated polyvinyl alcohol flag (4 mm x 6 mm, 20 μm in thickness and weighing 0.5 g), which is
attached to a paper-covered handle. When applied to the eye, the flag detaches and gradually dissolves, releasing the drug [100].

![Diagram of a N.O.D.S. device](image)

Fig.1.10. Schematic representation of a N.O.D.S. device [100].

Greaves *et al.* [16] carried out a gamma scintigraphic study (12 healthy volunteers) on the precorneal residence and pharmacodynamic action of a radiolabelled Novel Ophthalmic Delivery System (N.O.D.S.) congener (99m-Technetium-labelled diethylenetriaminepentaacetic acid) containing pilocarpine nitrate. Pilocarpine delivered by N.O.D.S. significantly decreased intraocular pressure as compared with pilocarpine delivered from a solution. The Novel Ophthalmic Delivery System (N.O.D.S.) also induced a greater decrease in pupil diameter as compared with the pilocarpine solution.

A study by Lawrenson *et al.* [100], which compared the corneal anaesthesia induced by different concentrations of proxymetacaine loaded Novel Ophthalmic Delivery System (N.O.D.S.) and conventional eyedrops, concluded that the varying Novel Ophthalmic Delivery System doses produced longer lasting anaesthesia than 35μl eyedrops containing more than three times the concentration of drug. The greater bioavailability achieved with this vehicle allowed the use of much lower drug concentrations, thus reducing the likelihood of adverse systemic reactions. However, although therapeutically effective, it has been shown that Novel Ophthalmic Delivery Systems (N.O.D.S.) are not as well tolerated by patients as conventional eyedrops [102].
Collagen shields were originally developed as soluble bandage contact lenses that would promote corneal healing [16,103]. Collagen is a structural protein of bones, tendons, ligaments and skin and comprises more than 25% of the total body protein in mammals. The shields, with a diameter of 14.5 mm and a thickness of about 1.0 mm in the centre, are composed of porcine scleral collagen [96]. Exposure to ultraviolet light for different time periods induces varying degrees of crosslinking, which results in shields with a range of dissolution rates. The collagen shields currently on the market do not contain drugs, but there have been many studies on their use as delivery systems for ophthalmic therapeutics [105]. For drug delivery, the shields are typically rehydrated in an aqueous solution of the drug, whereby the drug is absorbed by the protein matrix and then released as the shield dissolves in the eye. Drugs that have been investigated for delivery using collagen shields include antibacterial agents, (tobramycin [104, 106] and gentamicin [107]), immunosuppressive agents (Cyclosporine A [108]), anti-inflammatory agents (dexamethasone [109]) and antiviral agents (trifluorothymidine [110]). O'Brien et al. [104], compared collagen shields with soft contact lenses in pharmacokinetic studies of the ocular penetration of tobramycin in rabbits. They compared three groups: animals receiving collagen shields rehydrated in 3 mg / ml tobramycin; animals receiving therapeutic soft contact lenses; and animals receiving topical tobramycin drops. Each group received tobramycin eyedrops (3 mg / ml) every five minutes for a total of six doses. Aqueous humour samples were taken 15 and 60 minutes following the last dose. At both times, the aqueous humour contained a significantly greater concentration of drug after administration with collagen shields than with either contact lenses or topical drops. Despite some promising experimental results, however, several disadvantages of collagen shields have been identified. The cornea must be anaesthetised before the shield is applied and, in contrast with medicated contact lenses, collagen shields often produce discomfort
and interfere with vision. In addition, there has yet to be a study that shows prolongation in ocular absorption of a drug beyond 30 minutes. As patients are unable to apply the shields without the aid of a physician, collagen shields are not considered to offer a significant advantage over other delivery devices [86].

Ocuserts are insoluble inserts used in the treatment of glaucoma [75,111]; these have been developed and marketed by Alza Corporation, USA, and are available in two forms: Pilo-20 and Pilo-40, which, respectively, are designed to release either 20 or 40 μg / h of pilocarpine for seven days when placed under the eyelid. The Ocusert is a thin, flexible elliptical device consisting of three layers, Fig. 1.11. The two outer layers are comprised of ethylene-co-vinylacetate and enclose an inner core of pilocarpine gelled with alginate; these are the rate-limiting membranes, i.e. they control the rate of drug release into the eye. A retaining ring of ethylene-co-vinylacetate, impregnated with titanium dioxide, as a marker of visibility, encloses the drug reservoir circumferentially. The dimensions of the Ocusert are 13.4 mm × 5.7 mm, with a thickness of 0.3 mm. The membranes are the same in both Pilo-20 and Pilo-40, but the reservoir of the Pilo-40 system contains about 90 mg of di(2-ethylhexyl)phthalate as a flux enhancer [96].

Fig.1.11. The Ocusert device [96].
Friedrich [112] carried out a study to evaluate Ocusert in 14 patients with ocular hypertension or glaucoma. It was found to be safe, well tolerated and as effective in lowering intraocular pressure as pilocarpine solution (2 %), lasting for at least 4 days. Edwards [40] investigated the suitability of Ocusert for the treatment of patients suffering from primary acute angle closure glaucoma. In two separate controlled studies, patients diagnosed with primary acute angle closure glaucoma were randomised to receive Ocusert Pilo-40 and either an intensive pilocarpine regimen or a low-dose pilocarpine treatment. All patients also received Diamox (acetazolamide) 500 mg i.v., as is usual in the treatment of primary acute angle closure glaucoma. Two hours after starting topical treatment, the study was terminated and ocular and systemic response to treatment and the outcome of treatment were assessed. Over the treatment period, a comparable reduction in intraocular pressure was observed in all three groups. The major advantages of Ocusert over other devices included longer duration of action, avoidance of accommodative spasms in younger patients and better patient compliance, particularly in those who fail to administer eyedrops due to inconvenience or forgetfulness, and in patients treated by another person; 20 % of all patients treated with Ocusert lost the device without being aware of it. In a small number of cases, leakages from the insert were suspected and, rarely, a spontaneous dislocation of the device in front of the pupil occurred, with corresponding sight impediment [8]. Heller et al [113] investigated, as a means of delivering 5-fluorouracil, an anti proliferative agent used as an adjunct to glaucoma filtering surgery. Release of 5-Fluorouracil from a crosslinked poly(orthoester) occurred predominantly by diffusion with little weight loss, while release of 5-Fluorouracil from a linear polymer occurred by an erosion-controlled process confined predominantly to the surface layers.

The minidisc or ocular therapeutic system was developed by Bawa et al. [114]. It consists of a 4-5 mm diameter contoured disc having a convex front and a concave back.
surface; it is placed in contact with the eyeball, and resides behind the lower or upper eyelid. The minidisc may be hydrophilic, where the major component is polyhydroxymethyl methacrylate, or hydrophobic, prepared from a silicone based prepolymer, \( \alpha,\omega\)-bis(4-methacryloxybutyl)-polydimethylsiloxane). *In vivo* tests, using albino rabbits, showed that the hydrophilic minidisc released sulfisoxazole for 118 h, while the hydrophobic unit released gentamicin sulphate for more than 320 h [96]. Comfort studies were carried out in 120 human eyes to identify the optimum curvature of the minidisc. Handling and insertion studies were also conducted for the purpose of comparing the minidisc with another commercially available preparation, the Lacrisert. The Lacrisert is a rod-shaped device made from hydroxypropyl cellulose; introduced by Merck Sharp and Dohme in 1981. The device, which weighs 5 mg and measures 1.27 mm in diameter with a length of 3.5 mm, has been used in the treatment of dry eye syndrome as an alternative to artificial tears: 24 subjects, of varying ages, were asked to insert the minidisc and the Lacrisert, both prepared, in this instance, from hydroxypropyl cellulose. The mean time required for patients to insert the Lacrisert was 39.5 s as compared with 16.6 s for a minidisc of the same mass. The patients were allowed 3 attempts at inserting both devices and, while only 18 were able to insert the Lacrisert, all 24 patients managed to insert the minidisc [96].

Other groups have described similar systems: Saettone *et al.* [8] compressed coated ophthalmic mini-tablets for the controlled delivery of timolol maleate. Two types of tablet were prepared, containing 0.34 mg (type 1) and 0.68 mg (type 2) timolol maleate, respectively. The tablets (3.5 mm in diameter, 1.5 mm in thickness) had an average mass of 13.0 mg (type 1) or 16.0 mg (type 2), and were prepared from varying proportions of hydroxypropyl cellulose and glyceryl palmito-stearate and coated with acrylic polymers Eudragit®-RS and Eudragit®-RL. It was found that the type and amount of acrylic polymer
coating could control drug release adequately: an adjustment of 11.1 % Eudragit RS/RL (80:20 w/w) to 17.8 % of the total weight of the tablet increased the release time from 25 hours to 70 hours [115]. Di Colo et al. [116] described the in vitro and in vivo release of ofloxacin from inserts (6 mm diameter, 20 mg) prepared from a mixture of high molecular weight (400 kDa) linear poly(ethylene oxide) and Eudragit L100. Inserts were also prepared using poly(ethylene oxide) and partially neutralised Eudragit. The insert erosion rate has been reported as being related to the strength of interpolymer interactions, and also to the hydrophilic-hydrophobic balance of the constituent polymers. In an in vivo study, the maximal concentration (C_max), the area under the curve (AUC), and the effective time (teff) of concentration above Minimum Inhibitory Concentration (MIC) were very similar for all commercial ofloxacin eyedrop controls, but were significantly increased by the use of plain poly(ethylene oxide) (PEO) inserts. This increase in bioavailability was attributed to the mucoadhesive properties of poly(ethylene oxide) and to increased tear fluid viscosity. In a later study, Di Colo et al. [116], investigated the effect on molecular weight of poly(ethylene oxide) on C_max and AUC: poly(ethylene oxide) 400 and poly(ethylene oxide) 900 showed increases in C_max of 3.78- and 3.16-fold, respectively, and in AUC of 11.06- and 12.37-fold, respectively, relative to commercial eyedrops. poly(ethylene oxide) 200 induced smaller increases, whereas poly(ethylene oxide) 2000 was unsuitable due to excessive swelling.

Alani first described an ophthalmic rod in 1978 [117]. The rod is made of an acrylic plastic and is 50 mm in length. Drug, which is present as a thin layer at one end of the rod, is loaded by dipping into drug solution. Pulling down the lower eyelid and rubbing the tip of the rod against the palpebral conjunctiva (2-3 seconds) achieves delivery of the drug. The thin dry layer of the drug is dissolved by the tears and buffered to pH 7.4. In a clinical study, [118], clonidine ophthalmic rods (20 μg) were used by 15 patients with glaucoma.
for 12 days. A regimen of three rods per day was sufficient to keep the intraocular pressure under control; 14 of the patients preferred the rods to eyedrops.

Gurtler et al. [119, 120] investigated soluble bioadhesive ophthalmic drug inserts containing gentamicin. Ophthalmic inserts based on mixtures of hydroxypropyl cellulose, ethyl cellulose, poly(acrylic acid) and 25.0% w/w gentamicin sulphate were manufactured, along with similar inserts prepared with cellulose acetate phthalate as an additional component. The inserts produced were rod shaped (2.0 mm x 5.0 mm, 22.5 mg) and consisted of hydroxypropylcellulose (40.2%), ethylcellulose (18.0%), carbomer (1.8%), cellulose acetate phthalate (15.0%) and gentamicin sulphate (25%). In vivo assays in rabbits and dogs showed that the inserts maintained therapeutic gentamicin levels over 72 h. In a later study, Baeyens et al. [121] further modified the release properties of bioadhesive ophthalmic drug inserts by premixing the gentamicin sulphate in an acetonic medium to form a gentamicin sulphate / cellulose acetate phthalate solid dispersion and by coating gentamicin sulphate / ethyl cellulose granules with Cellulose Acetate Phthalate (CAP) to form a gentamicin sulphate / ethyl cellulose / cellulose acetate phthalate co-precipitate. In addition hydroxypropylmethylcellulose (HPMC), a less hydrophilic polymer than hydroxypropylcellulose, was used as a vehicle constituent. Inserts containing gentamicin sulphate / cellulose acetate phthalate solid dispersion, gentamicin / ethyl cellulose / cellulose acetate phthalate co-precipitate and hydroxypropylmethylcellulose displayed improved efficacy as compared with inserts containing gentamicin sulphate that had not been subjected to pretreatment. A high irritation level was observed for inserts containing the gentamicin / ethyl cellulose / cellulose acetate phthalate and hydroxypropylmethylcellulose; a relationship between $t_{eff}$ and irritation score was established, emphasising the importance of irritability scores for the evaluation of the potential of such systems.
Chetoni et al. [122] described the development and in vitro and in vivo testing of rod-shaped mucoadhesive ophthalmic inserts for the upper or lower conjunctival fornix. Cylindrical devices (0.9 mm × 6-12 mm, 3-8 mg) containing 0.8 mg oxytetracycline hydrochloride were prepared from appropriate mixtures of silicone elastomer, oxytetracycline hydrochloride, and sodium chloride as a release modifier. A stable polyacrylic or polymethylacrylic acid interpenetrating polymer network was grafted onto the surface of the insert. In vitro experiments showed that the polymethylacrylic acid-grafted devices released oxytetracycline hydrochloride at lower rates than those grafted with polyacrylic acid. When tested in rabbits, some polymer-grafted inserts maintained oxytetracycline hydrochloride concentrations in the lachrymal fluid to levels exceeding the minimum inhibitory concentration (MIC) for several days. The ocular retention of polymer-grafted inserts was significantly higher than that of the ungrafted inserts; this has been attributed to the mucoadhesive properties of the former.

There have been a number of studies in recent years on the use of Gelfoam® in ophthalmic drug delivery. Simamora et al. [123] evaluated one such ocular device, for the controlled delivery of pilocarpine in albino rabbits. The device was prepared by impregnating a section (2.5 mm × 2.5 mm × 1.0 mm) of Gelfoam® with a mixture of pilocarpine hydrochloride and cetyl ester wax in chloroform solution. In a crossover study, the efficacy of the device was compared with that of pilocarpine eyedrops and pilocarpine gel. The in vivo results showed that the Gelfoam® device was more effective than the two conventional pilocarpine dosage forms in prolonging the duration of the pilocarpine activity. Later, Lee and Yallowsky [92] investigated enhancer-free ocular devices based on Gelfoam® for the controlled systemic delivery of insulin; it is generally believed that ophthalmic solutions or devices for the systemic delivery of insulin require the addition of an absorption enhancer, usually a surfactant or chelating agent [90]. In this study,
Gelfoam® ocular devices containing 0.2 mg of sodium insulin were prepared in either water or 10 % acetic acid and were evaluated in rabbits. Results showed that blood glucose levels were significantly lowered after treatment with the acidified insulin-Gelfoam® device, but not with the water-treated device. The results suggested that a change in the Gelfoam® upon treatment with acid is responsible for the efficient systemic absorption of insulin from these enhancer-free devices. A recent study by Negvesky et al. [124] has investigated Gelfoam® discs as an alternative topical ophthalmic drug delivery system for effecting pupillary dilation in humans. Discs (4.0 mm x 0.5 mm) cut from sterile Gelfoam® using a hole punch were impregnated with a mixture of tropicamide and 1-phenylephrine hydrochloride. After drying, a disc was placed in the inferior fornix of a randomly selected eye and the fellow eye was treated with a drop each of phenylephrine hydrochloride solution and tropicamide solution. The Gelfoam® treated eyes showed a median change in dilation diameter that was approximately 25 % greater than that for the topically treated fellow eyes.

Diestelhorst et al. [102] described a preservative-free ophthalmic delivery system known as "Dry Drops". The active ingredient is incorporated in a drop of hydrophilic polymer solution that is freeze-dried on the tip of a soft hydrophobic carrier strip. On contact with the tear film or conjunctiva, the lyophilisate immediately rehydrates and detaches from the carrier. Dry Drops containing hydroxypropylmethylcellulose as the active ingredient and a preservative-free tear substitute were applied to the eyes of 32 healthy volunteers, and tolerability and safety were quantified using slit-lamp biomicroscopy, questionnaire and visual analogue scales. Although there were no statistical differences between Dry Drops and the conventional formulation, the initial sensation was slightly less uncomfortable than that of the solution control. The authors
claim that other advantages of this system include improved chemical stability, exact dosing and reduced risk of lesions to the eye surface.

1.5.2.6. Prodrugs

Prodrugs are pharmacologically inactive derivatives that are chemically or enzymatically converted to their active parent compound after administration [125]. The principle of ocular prodrugs is to enhance corneal drug permeability by modifying the lipophilicity or hydrophilicity of a drug. Most ophthalmic drugs contain functional groups such as alcohol, carboxylic acid and phenol, which lend themselves to simple derivatisation. Prodrug derivatisation of drug molecules that target various nutrient transporters (which include peptide, amino acid, folate, monocarboxylic acid transporters and so on, that have been reported to be expressed on the retina and blood-retinal barrier) could result in enhanced ocular bioavailability [126]. An ideal prodrug for ophthalmic delivery should be stable and soluble in aqueous solutions, be sufficiently lipophilic in order to penetrate through the cornea and be capable of releasing the parent drug in the eye at a rate that satisfies the therapeutic requirement. Bodor et al [129] suggested that real breakthrough in the area of ophthalmic therapeutics can be achieved only by specifically designing new prodrugs for ophthalmic applications to incorporate the possibility of eye targeting into their chemical structure.

Pilocarpine, that is widely used to treat patients with glaucoma, has a poor ocular bioavailability if administered topically [127]. Suhonen et al. [128] produced a series of prodrugs of pilocarpine esters that were hydrolysed by esterases after instillation to yield pilocarpine on the endothelial side of the cornea. These prodrugs showed a $2-7 \times$ higher permeability than pilocarpine despite their large molecular weights. Pilocarpic acid esters
are reported to be of particular interest as they combine enhanced bioavailability and prolonged duration of activity with high stability in eyedrop formulations [83].

Sasaki et al. [130] prepared lipophilic, acetylated prodrugs of the β-blocker tilisolol and investigated their in vitro ocular permeability and in vivo absorption in rabbits. All the derivatives considered showed increased lipophilicity, due to the introduction of a lipophilic group, and rapid enzymatic conversion to tilisolol in ocular tissue homogenates. Corneal penetration of lipophilic derivatives was found to be 3-6 x higher than that of tilisolol. It was concluded that the use of prodrugs in the delivery of β-blockers might help to overcome some of the problems associated with their systemic absorption when applied topically. Nonetheless, the usefulness of β-blockers in the treatment of glaucoma has been limited by the relatively high incidence of cardiovascular and respiratory side effects [131].

Although prodrugs have been proven to be of value, they are considered particularly demanding in terms of the extensive pharmacokinetic and pharmacological information required for their commercial development [10].

1.5.2.7. Ion pairs

Some anions and cations are able to form complexes (ion pairs) by intermolecular electrical interaction. Lipophilic ion pair formation has been proposed as an approach for the enhancement of the corneal penetration of drugs: the drug is paired with a counterion, which imparts appropriate properties of lipophilicity, physiological compatibility and stability. Wilson et al. [132] reported ion pair formation between sodium cromoglycate and dodecylbenzyltrimethylammonium chloride. The extent and rate of corneal penetration of both constituent ions was altered upon their co-administration. In particular, a marked increase in the concentration of both ions in the aqueous humour was observed after instillation of the ion pair formulation.
1.5.2.8. Iontophoresis

Iontophoresis is a non-invasive process for the delivery of ionic drugs, in which a direct current (1-2 mA) is used to drive the ions into cells or tissues. The technique was first introduced in the early 1900s for the transcutaneous administration of strychnine to rabbits, and has since been employed in ophthalmology for the treatment of corneal ulcers, keratitis and episcleritis [9]. An iontophoresis unit consists of a battery-operated variable power supply (0.2- mA, DC) and two electrodes. The drug is applied with an electrode carrying the same charge as the drug, while the ground electrode, which is of opposite charge, is placed elsewhere on the body to complete the circuit. The salt form of a drug is usually used as the dissociated salt is highly soluble in water and has a high charge density. The probe features an eyecup that is held in place by the partial vacuum obtained by withdrawing the plunger of the syringe. Transcorneal and transscleral iontophoresis have been shown to be effective. Transcorneal iontophoresis can deliver and sustain high concentrations of drugs into the anterior segment of the eye, particularly into the cornea and aqueous humour [133,135]. Hughes and Maurice [134] have found that transcorneal iontophoresis of gentamicin in the rabbit eye increased permeability to the antibiotic more than 100 ×, as compared to topical exposure in the absence of current. Transscleral iontophoresis has been used successfully to deliver gancyclovir, gentamicin and vancomycin into the vitreous body in sufficient doses for the treatment of such posterior segment diseases as cytomegalovirus retinitis and endophthalmitis [9]. Barza et al. [136, 137] reported the transscleral iontophoresis delivery of therapeutic concentrations of three antibiotics (cefazolin, ticarcillin and gentamicin) to the vitreous humour of uninfected rabbit eyes. This study showed that high concentrations of antibiotics could be delivered to the vitreous humour by a method that does not involve subconjunctival injection [137].
The advantages of iontophoresis over other drug delivery techniques include the minimisation of systemic exposure to the drug, delivery of the drug directly to the target tissue and improved consistency of drug penetration [134]. Maurice and Barza et al. [138] performed transscleral iontophoresis by placing the iontophoretic probe over the *pars plana* and were able to deliver high concentrations of drugs to the vitreous humor. However, although iontophoresis is an effective method of delivering ophthalmic drugs - including antibacterials, antivirals and antifungals - to the cornea, aqueous humour and vitreous body, it has not become popular since it requires the use of inconvenient equipment and has specific electrical requirements. Furthermore it has been shown that iontophoresis can cause damage to the ocular tissues, although this may be minimised by using the lowest current densities and shortest duration of treatment required for ensuring adequate penetration of the drug [139].

1.5.2.9. **Cyclodextrins**

Cyclodextrins are compounds used to complex drugs that have problems associated with issues such as poor solubility, instability, irritation to the target organ or area and difficulty in formulation. Complexing of some drugs with cyclodextrins can result in improved dissolution, wettability, stability, solubility and reduced side effects [140,141]. Cyclodextrins are a group of cyclic oligosaccharides with a hydrophilic outer surface consisting of six, seven or eight glucose units - α, β and γ-cyclodextrin, respectively - and although soluble in water, have a lipophilic cavity in the centre. Cyclodextrin molecules are relatively large, having molecular weights of between 1000 and 1500 and, under normal conditions, will only penetrate biological membranes with considerable difficulty [142, 143]. The central lipophilic cavity enables fat-soluble drugs to be taken up inside the cyclodextrin and so increases the solubility of such drugs. The cyclodextrins act as
penetration enhancers by ensuring a constant high concentration of dissolved drug at the membrane surface. Studies on the earliest cyclodextrins showed that they possessed nephrotoxicity and haemolytic activity; this limited their use in ophthalmic drug delivery [144]. Since then, new cyclodextrin derivatives such as 2-hydroxypropyl-\(\beta\)-cyclodextrin have been developed for use in ophthalmic drug delivery systems. Fridriksdottir et al. [145] investigated the effect of 2-hydroxypropyl-\(\beta\)-cyclodextrin solutions, mixed with the carbonic anhydrase inhibitor methazolamide, on the lowering of intraocular pressure in rabbits. Methazolamide, which has a limited aqueous solubility, is usually administered orally to treat glaucoma. The results of this study indicated that, in reducing intraocular pressure, methazolamide formulated in aqueous 2-hydroxypropyl-\(\beta\)-cyclodextrin solutions, was almost as effective as a 0.5 % w/v solution of timolol. Reer et al. [146] demonstrated that a formulation containing 2-hydroxypropyl-\(\beta\)-cyclodextrin, buffered in the range pH 6.5-7.0, increased the in vitro permeability of diclofenac sodium through the pig cornea. On the basis of this work it was suggested that such a formulation would be suitable for the dissolution, stabilisation and ocular administration of diclofenac eye drops. Nonetheless, although cyclodextrins seem to be beneficial for poorly water-soluble drugs, they do not seem to afford a real improvement for hydrophilic drugs, except in eliminating ocular irritation in cases where this has proved to be a problem.

1.5.2.10. Mucoadhesive polymers and in situ gelling systems

Bioadhesive / mucoadhesive vehicles

There has been much interest in the use of mucoadhesive polymers as a means of prolonging contact between the drug instilled and the corneal epithelium. A suitable mucoadhesive polymer for use in the eye is one that will attach to corneal mucin, via non-covalent bonds, and remain in contact with precorneal tissues until mucin turnover causes
elimination of the polymer.

Bioadhesion (see section 1.6 for more details) may be described as the attachment of synthetic or natural polymers to a biological substrate. The term mucoadhesion is used if the substrate is mucous or a mucous membrane. Mucoadhesives are usually polymers with numerous hydrophilic functional groups that are capable of forming hydrogen bonds, for example carboxyl, hydroxyl, amide and sulphate groups. Hydrogen bonding appears to play a significant role in mucoadhesion, as does the presence of water [147].

Mucoadhesive polymers used in ocular drug delivery are placed in the front of the eye and attach themselves through non-covalent interactions to the mucin that covers the conjunctiva and cornea. Water-soluble mucoadhesive polymers dissolve slowly and are removed by tears while water-insoluble ones remain attached until the mucin replaces itself or until they are dislodged by blinking [82]. The use of mucoadhesive polymers in ophthalmic drug delivery may help to prolong contact between drugs incorporated into the formulation and the corneal and conjunctival epithelia and, hence, may facilitate improved delivery of the drug into the ocular tissues [148,149,150]. Examples of mucoadhesive polymers include hyaluronic acid, polyacrylic acid hydrogels and chitosan.

Hyaluronic acid is a glycosaminoglycan that is present in some bacterial capsules, the intercellular matrix of the connective tissue of most vertebrates, and also in the vitreous and aqueous humours of the eye. The molecule is a linear polydisaccharide of the form - (G-N)n-, in which G is glucuronic acid and N is N-acetylglucosamine. Sodium hyaluronate products are already widely used in intraocular surgery. Investigations into the use of sodium hyaluronate as an ophthalmic drug delivery system have shown that sodium hyaluronate can increase the precorneal residence time of certain drugs in humans, and that low concentrations of the polymer (< 0.1 %) are particularly effective [59,151]. Saettone et al. [152] investigated the mucoadhesive properties and in vivo activity of ophthalmic
vehicles based on hyaluronic acid. The hyaluronic acid preparations were found to have good mucoadhesive properties; when tropicamide was incorporated into the hyaluronic acid formulations, these were capable of increasing the area under the mydriasis curve by up to $4 \times$ compared with polyacrylic acid formulations.

Polyacrylic acids are available in a range of molecular weights and may be branched, linear or crosslinked: Carbopol 934P is appropriate for use in the pharmaceutical industry: it is lightly crosslinked through co-polymerisation with allyl sucrose or allyl pentaerythritol, has a molecular weight of $ca. 3.0 \text{ MDa}$ and is soluble in aqueous media. Carbopol 934P has several advantages over other vehicles in that it is generally more comfortable than inserts and is instilled like an ointment without significant blurring of vision. Smart et al. [153] investigated the adhesiveness of various materials to mucus and found polyacrylic acid to possess good mucoadhesive properties. Hui and Robinson [154] investigated the ocular delivery of progesterone using polyacrylic acid. Acrylic acid crosslinked with divinyl glycol and 2,5-dimethyl-1,5-hexadiene showed excellent bioadhesion to conjunctival mucin, as determined by visual inspection and an in vitro method using the force required to separate a polymer specimen from a freshly excised rabbit conjunctival membrane. Davies et al. [64] evaluated the potential of Carbopol 934P as an ophthalmic vehicle in the rabbit and found that precomeal clearance of Carbopol was significantly lower than for polyvinyl alcohol. Thermes et al. [155] compared the effect of poly(acrylic acid) on the ocular bioavailability of timolol using 0.5% aqueous Timoptol or isoviscous solutions of polyvinyl alcohol as controls. Polyacrylic acid formulations extended the concentration versus time profiles of timolol and gave the highest timolol concentrations in the iris and ciliary body.

Chitosan has been evaluated by a number of workers for both its mucoadhesive properties and its suitability as a drug delivery vehicle [156,158,160,161]. Chitosan is a
natural polyaminosaccharide and is composed of deacylated chitin, a cellulose-like biopolymer consisting predominantly of unbranched chains of $\beta$-(1→4)-2-acetamido-2-deoxy-D-glucose. It is obtained from the exoskeleton of marine invertebrates and arthropods and isolated industrially by hydrolysing the aminoacetyl groups of chitin from crabs or shrimps in aqueous alkaline solutions. Lehr et al. [157] and Henriksen et al. [159] have reported that chitosan possesses fairly good mucoadhesive properties. Park and Robinson [161] and Lehr et al. [157] suggest that, in neutral or slightly alkaline conditions as would be desirable for adhesion in the eye, cationic polymers (such as chitosan) are likely to be superior mucoadhesives. Felt et al. [162] evaluated the ocular tolerance and precorneal retention of chitosan. An ocular irritation test, using confocal laser scanning ophthalmoscopy combined with corneal fluorescein staining, showed excellent tolerance of chitosan in rabbits. In parallel, gamma scintigraphic data showed that the clearance of $^{99m}$Tc-diethylenetriaminepentaacetic acid was significantly delayed in formulations incorporating chitosan; at least a three-fold increase of the corneal residence time was achieved in the presence of chitosan as compared with Tobrex® formulations.

In situ gelling systems

In situ gelling systems have been developed in an attempt to overcome some of the problems associated with the delivery of ophthalmic drugs. These systems are more acceptable to patients since they are conveniently dropped into the eye, typically as a solution, after which they undergo transition into a gel [163]. Studies have shown that the precorneal residence times of some in situ gelling systems last for several hours [61, 154]. The change in viscosity that characterises these systems can be due to a change in pH, temperature or ionic strength and leads to an increase in drug bioavailability by slowing nasolacrimal drainage [10,164]. The sol-to-gel phase transition results from a competitive balance between a repulsive force that expands the polymer network and an attractive force.
that contracts the network. The most effective repulsive force that can be imposed upon a phase-transition polymer is the electrostatic interaction between the polymer charges caused by introducing ionisation into the network. The osmotic pressure created by the counterion adds to the expanding pressure. The attractive forces can be van der Waals, hydrophobic interaction, ion-ion electrostatic interaction and/or hydrogen bonding [165].

Polymers that are pH-sensitive possess charges or ionisable groups that are covalently attached to a macromolecular backbone; these are either polymeric acids, such as those containing carboxylic acid, or bases such as those containing primary amines. Such polymers ionise reversibly over an inherent pH range. The resultant charge facilitates the swelling of the polymer network and renders the matrix permeable to water-soluble solutes [165]. Typical polymers used as pH-sensitive systems are lightly crosslinked acrylic acid polymers, which have an extensive capacity to attract and hold water.

Examples of in situ gelling systems include cellulose acetate phthalate latex, crosslinked polyacrylic acid and derivatives, such as polycarbophil and carbomers, which all undergo a phase change when pH varies. Poloxamers, methylcellulose and Smart Hydrogel™, a graft co-polymer of poloxamer and polyacrylic acid, all exhibit a phase change due to a change in temperature. Gelrite and alginate are polymers that undergo a phase change due to a change in ionic strength. Kumar et al. [166] investigated the rheological characteristics of an in situ gelling system consisting of a combination of Carbopol and methylcellulose. The study showed that a solution containing 1.5 % methylcellulose and 0.3 % Carbopol at pH 4.0 and 25 °C formed a strong gel at pH 7.4 under simulated physiological conditions.

Paulsson et al. [167] investigated the effect of temperature and that of various ions on gel formation characteristics of gellan gum in vitro; gellan is an anionic polysaccharide composed of tetrasaccharide repeating units. The influence of the various ions present in
tear fluid (Na⁺, K⁺ and Ca²⁺) were considered in terms of their effects on gel strength and the consequences of dilution due to reflex tearing were considered. Na⁺ was reported to be the most important gel-promoting ion *in vivo*.

Gurney *et al.* [151] conducted a comparative study of the miotic response induced by a pH-sensitive dispersion, a temperature setting gel and hyaluronic acid all formulated with pilocarpine. This group of workers reported that formulations containing hyaluronic acid induced the highest miotic response in man whereas the *in situ* gelling systems are washed off the corneal surface as miotic activity could be measured for only ten minutes following administration.

Rozier *et al.* [168] investigated the ocular absorption of timolol administered in Gelrite, or in an equiviscous solution of hydroxyethylcellulose; the albino rabbit model was employed for both sets of experiments. In both cases, Timolol was detected in the cornea for up to two hours, but a significantly higher corneal content of timolol is reported for all the Gelrite formulation at all time points.

Although certain gelling systems have been shown to improve precorneal retention and the bioavailability of ocular drugs, the benefits are of limited value as once- or twice-a-day dosing is the typical expectation from these systems.

**1.6. Bioadhesion and mucoadhesion**

The concept of mucosal adhesives was first introduced in the early 1980s. Mucoadhesives are synthetic or natural polymers that interact with the mucus layer covering a mucosal epithelial surface. The concept of mucoadhesion has alerted many investigators to the possibility that such polymers may be used to overcome the
physiological barriers in sustained drug delivery and to facilitate prolonged contact between a drug and its target tissue.

Bioadhesion may be defined as the regime at which two materials, at least one of which is of biological origin, are held together for extended periods of time [169]. In the context of drug delivery, the term 'bioadhesion' refers to the adhesion of synthetic or biological macromolecules to biological tissue. The biological substrate may be epithelial tissue, or it can be the mucus that is coating the surface of a tissue. If adhesive attachment is to a mucous coating, the phenomenon is referred to as mucoadhesion [147]. Mucoadhesive drug delivery systems utilise hydrophilic polymers that become bioadhesive on mild hydration; such systems may be used for targeting drugs to mucosal regions of the body.

1.6.1. Mechanisms of mucoadhesion

Mucus is a naturally occurring bioadhesive, which is present as either a gel layer adherent to the mucosal surface or in a luminal, soluble, or suspended form, depending on its location in the body. Mucus binds to the surface of cells and exhibits adhesive properties toward a variety of substrates. The attachment of mucin to the cell surface is the result of a non-covalent interaction between a macromolecule and mucin. In many descriptions of the interactions between mucoadhesive materials and a mucous membrane, two discrete steps are identified: the contact stage, during which intimate contact is established between the mucoadhesive and mucous membrane, is followed by the consolidation stage, at which various physicochemical interactions operate synergistically to consolidate and strengthen the adhesive joint [169].

Mortazavi and Smart [170] have reported that, on application of a constant tensile stress to compacts of mucoadhesive polymers, a cohesive failure of the swelling polymer
occurs for all but the weakest adhesives. Various theories have been put forward to account for the cohesive mechanism operating between the mucin and mucoadhesive material.

1.6.2. Theories of mucoadhesion

Amongst the number of theories have been proposed to explain the fundamental mechanisms of adhesion [171, 172], five have been adapted to the study of bioadhesion. Some of these theories consider the formation of mechanical bonds whereas others focus on chemical interactions, they will be briefly described below [173].

1.6.2.1. Electronic theory

The hypothesis of the electronic theory relies on the assumption that the bioadhesive material and the target biological substrate have different electronic structures. Electron transfer occurs when the two materials come into contact, resulting in a double layer of electrical charge at the bioadhesive-biological substrate interface. The bioadhesive interaction is believed to be due to the attractive forces across this double electrical layer.

1.6.2.2. Adsorption theory

According to the adsorption theory, the bioadhesive bond formed between an adhesive substrate and tissue or mucosal is due to van der Waals' interactions, hydrogen bonds and electrostatic forces. Although these forces are individually weak, the large number of interactions is able to produce intense adhesive strength. The adsorption theory is the most widely accepted of the five theories of adhesion.
1.6.2.3. Wetting theory

The wetting theory is predominantly applicable to liquid bioadhesive systems and uses interfacial tensions to predict spreading over a biological substrate and, in turn, adhesion. The contact angle (θ) between a liquid and a biological substrate should be close to zero for proper spreading. It is related to interfacial tensions (γ) through Young's equation. The expression for bioadhesive gel spreading over soft tissue in the gut, may be written as:

\[ \gamma_{tg} = \gamma_{bt} + \gamma_{bg} \cos \theta \]

where the subscripts t, g, and b stand for tissue, gastrointestinal contents and bioadhesive polymer, respectively. For spontaneous wetting, the contact angle must be equal to zero, i.e.:

\[ \gamma_{tg} = \gamma_{bt} + \gamma_{bg} \]

The work of adhesion (Wa) is an important parameter that may indicate the strength of an adhesive bond. It is expressed in terms of surface and interfacial tension (γ), and is defined as the energy per unit area that is released when an interface is formed. The work of adhesion is given by:

\[ W_a = Y_A + Y_B - Y_{AB} \]

where the subscripts, A and B, refer to the biological membrane and the bioadhesive formulation respectively.

1.6.2.4. Diffusion theory

The diffusion theory supports the concept that the interpenetration and entanglement of bioadhesive polymer chains and mucus molecules produces semi-permanent adhesive bonds. It is thought that bond strength increases with the degree of penetration of the polymer chains into the mucus layer. The depth to which the polymer
chains penetrate the mucus depends on the diffusion coefficients and concentration gradients of the materials and the time of contact between them. The diffusion coefficient, in turn, depends on the degree of crosslinking of the polymer. The macromolecular penetration effect, where the mucoadhesive molecules interpenetrate and bond by secondary interactions with mucus glycoproteins, is a theory that has received considerable support. Jabbari et al. [174] have found that when a mucin solution is placed into contact with a thin crosslinked film of polyacrylic acid formed on an attenuated total reflectance crystal, the attenuated total reflectance (ATR-FTIR) spectra reveal a peak at 1550 cm\(^{-1}\), which is attributed to mucin dimeric carboxylic C=O stretching. It was proposed that this indicated the presence of interpenetrating mucin molecules within the polyacrylic acid film.

1.6.2.5. Fracture theory

The fracture theory analyses the forces required to separate two adhered surfaces after adhesion. The maximum tensile stress \((s_m)\) associated with detachment can be determined by dividing the maximum force of detachment \(F_m\) by the total surface area \((A_0)\) involved in the adhesive interaction:

\[
s_m = \frac{F_m}{A_0}
\]

The fracture strength \(\sigma\), equivalent to the bioadhesive bond strength, may be calculated from:

\[
\sigma \equiv \sqrt{\frac{E \varepsilon}{L}}
\]

where \(E\) is Young’s modulus of elasticity, \(\varepsilon\) is the fracture energy and \(L\) is the critical crack length upon separation of the two surfaces [172]. Thus, the stiffness (elastic modulus) of the material can be used as a measure of bioadhesion.
1.6.3. Factors affecting mucoadhesion

The bioadhesive strength of a polymer may be affected by several factors, as discussed below.

1.6.3.1. Nature of the polymer

Studies have shown that there is a critical polymer molecular weight at which bioadhesion is at a maximum. According to Gurny et al. [175] the bioadhesive force increases with average molecular weight of the polymer up to around 0.1 MDa but beyond this value there is no further influence on the bioadhesive effect. Smart et al. [176] have found that the average molecular weight of sodium carboxymethylcellulose must exceed 78.6 kDa if this material is to exhibit significant bioadhesion. In contrast to the findings by Gurny et al. [175], studies by Duchene and Ponchel [177] and also by Mortazavi and Smart [176] have revealed that polyacrylic acid with an average molecular weight of 0.75 MDa is the most bioadhesive amongst this class of materials. Polyethylene oxide, a polymer containing molecules of highly linear configuration, exhibits an increase in adhesive strength up to an average molecular weight of 4.0 MDa. By contrast, dextrans with an average molecular weight of 19.5 MDa have been found to possess similar bioadhesive strength as those with a molecular weight of 0.2 MDa [172]. The flexibility of polymer chains is important in facilitating interpenetration and entanglement. The higher the chain mobility, the greater the interdiffusion and the more efficient the penetration of the polymer into the mucus network [177,178]. Park and Robinson [179] found that on lowering the concentration of the crosslinking agent, the mucoadhesive strength of polyacrylic acid increased. Increased crosslinking density also resulted in a decrease in swelling of the polymer; attributed to a reduction in chain mobility [180].
1.6.3.2. Swelling and hydration

Bioadhesive polymers in aqueous media hydrate and swell to form a gel. The strength of adhesion of such polymers has been shown to reach a maximum at an optimum degree of hydration [181]. The type and number of hydrophilic groups within the polymer structure influences the extent and rate of hydration of the polymer; these commonly include carboxyl, hydroxyl, amide and sulphate groups, all of which are capable of participating in hydrogen bond formation. The presence of water leads to partial hydration of the polymer and increased availability of the adhesive sites for secondary bond formation. However, large volumes of water can lead to the formation of a slippery, non-adhesive mucilage at the bioadhesive interface as a result of the incorporation of excessive amounts of water within the hydrogen-bonded network [182].

1.6.3.3. Effect of the pH of surrounding medium

Studies have shown that the bioadhesive properties of some polymers are affected by the pH of the surrounding media. Ch'ng et al. [183] observed that the pH of the medium is critical in determining the degree of hydration of highly crosslinked poly(acrylic acid)s: hydration was seen to increase with increasing pH, up to pH 7. This was attributed to differences in charge density at different pH levels and suggests that mucoadhesion is favoured when the carboxylate groups are in the unionised form; at pH of about 4, around 80% of the carboxylate ions of the polyacrylic acid are in the unionised form. Park and Robinson [179] investigated the mucoadhesive strength of polycarbophil at various pH values. This polymer showed maximum adhesive strength at pH 3 with a gradual decrease in adhesive strength as the pH increased to 5; the material failed to exhibit any mucoadhesive property above pH 5. This study showed that it is the protonated, rather than
ionised carboxyl groups, that interact with mucin molecules to promote mucoadhesive bonding.

1.6.3.4. Contact time and forces applied

Park and Robinson [179] showed that the strength of adhesion of poly(acrylic acid-co-divinylbenzene) and poly(hydroxyethyl methacrylate) to a mucin substrate can increase with the force applied or with the duration of application. In parallel, Leung and Robinson [147] postulated that mucoadhesion is a time dependent process. The same workers showed the importance of openness of the interacting networks with regard to mucoadhesion, and as a result suggested that interdiffusion is a process of paramount importance. According to these workers, an increase in the openness of the network can lead to an increase in interpenetration between the substrate and the mucoadhesive. This, in turn, results in an increase in contact surface area and mechanical entanglement with a consequent increase in mucoadhesive strength. If interdiffusion is important in mucoadhesion, the mean diffusional path ($s$) will be related to the diffusion coefficient ($D$) and time ($t$), according to:

$$s = \sqrt{2Dt}$$

However, experimental evidence showed that the mucin-mucin and polymer-mucin tensile strengths are both time dependent [184]. Furthermore, the effect of additional factors needs to be considered. These include: mucin turnover [180], temperature [184], formulation [155, 185], metal ions [186] and concentration of polymer [172, 180].
1.7. Techniques used for physical characterisation

1.7.1. Swelling measurements

The swelling experiments described in this thesis examine the swelling characteristics of a number of mucoadhesive and non-mucoadhesive polymers in order to identify and select those materials which display limited but rapid swelling for further investigation. It is deemed important that in order to prolong retention and minimise discomfort and disruption of vision, the polymers chosen for further study must swell quickly but to a limited degree.

Swelling controlled release systems may be capable of delivering drugs at constant rates over an extended period of time. In such systems, the rate of drug delivery is controlled by drug (solute) diffusion across a concentration gradient, by polymer relaxation (occurring as the crosslinked polymer takes up water), and by osmotic pressure variations during the swelling process. Swelling controlled release systems are valuable due to the possibility of achieving zero-order release [187].

In swelling-controlled polymeric systems the drug is usually dissolved or dispersed in the polymer. The solvent is then removed to give a solvent-free glassy polymeric matrix that contains the dispersed drug. In a drug release experiment, there is no drug diffusion through the unhydrated solid phase. As the dissolution medium (usually water, saline or biological fluid) penetrates the matrix, the polymer begins to swell. If the thermodynamic compatibility of the dissolution medium with the polymer is favourable, the glass transition temperature ($T_g$) of the polymer becomes lowered. Under these conditions, the swollen polymer converts to the rubbery state and the dispersed drug is able to diffuse outward.

Two interfaces, or fronts, are characteristic of this type of swelling behaviour: a front separating the glassy from the rubbery state (swelling interface), which moves toward
the glassy state with velocity $v$; and a front separating the rubbery polymer from the pure
dissolution medium (polymer interface), which moves outward (see Fig.1.12).

Fig. 1.12. Moving fronts during
dynamic swelling of a glassy
polymer. A, glassy state; B,
rubbery state; C, dissolution
medium; S, swelling interface; P,
polymer interface [188].

If no physical or molecular restrictions to swelling are present, the polymer will
eventually dissolve. It is possible to formulate matrices that do not dissolve, by employing
semi-crystalline or amorphous polymers that are slightly crosslinked. Such systems are
known as swellable, non-erodible release systems. In other systems, dissolution of the
polymer may occur due to chemical degradation, such as hydrolysis; such materials are
known as swellable, erodible release systems. Controlled release matrices that are
susceptible to enzymatic biodegradation are known as swellable, bioerodible release
systems.

There have been a number of experimental and theoretical investigations of solvent
and solute transport in polymeric systems, with several mathematical models developed to
describe transport behaviour. Fickian or non-Fickian drug diffusion mechanisms may be
observed depending on the dynamics of polymer swelling, the relative mobility of the drug
and the dissolution medium. Transport phenomena conforming to the Fickian mechanism
(also known as Case I diffusion) represent simple diffusion of solute down a concentration gradient. Fick's first law relates the flow of material through a concentration gradient:

\[ J = -D \frac{dc}{dx} \]

Where \( J \) is the flux of a component across a plane of unit area and \( D \) is the diffusion coefficient, \( c \) is the concentration and \( x \) is the distance travelled. The negative sign indicates that the flux is in the direction of decreasing concentration. \( J \) is in mol m\(^{-2}\) s\(^{-1}\), \( c \) is in mol m\(^{-3}\) and \( x \) is in m and the units of \( D \) are m\(^2\) s\(^{-1}\). [189]

It is known that the diffusion of the solvent is linked to the physical properties of the polymer network and the interactions between the polymer and the solvent. The amount of solvent absorbed per unit area of polymer at time \( t \), \( M_t \), is represented by

\[ M_t = k t^n \]

where \( k \) is a constant and \( n \) a parameter (the value of which lies between \( \frac{1}{2} \) and 1) related to the diffusion mechanism. This equation can be used to describe solvent diffusional behaviour for any polymer-solvent system whatever the temperature and solvent activity.

Fickian diffusion is often observed in polymer networks at temperatures well above the glass transition temperature of the polymer. If the polymer is in the rubbery state, the polymer chains have a high mobility that allows the solvent to penetrate the bulk material more easily. Thus, Fickian diffusion is characterised by a solvent diffusion rate that is slower than the polymer relaxation rate. The solvent concentration profile shows an exponential decrease from the completely swollen region to the core of the polymer. The diffusion distance is proportional to the square root of time:

\[ M_t = k \sqrt{t} \]

where \( M_t \) is the amount of drug released at time \( t \) and \( k \) is a constant.

Non-Fickian diffusion processes are mainly observed in glassy polymers, below \( T_g \). At a specific temperature below \( T_g \), the polymer chains are not sufficiently mobile to
permit immediate penetration of the solvent into the polymer core [190]. Two types of non-Fickian diffusion have been defined: Case II diffusion, and anomalous diffusion. The main difference between the two types concerns the solvent diffusion rate. In Case II diffusion, the solvent diffusion rate is faster than the polymer relaxation process, whereas in anomalous diffusion the solvent diffusion rate and the polymer relaxation are of about the same order of magnitude [191].

Case II diffusion may involve a number of alternative scenarios. There may be a rapid increase in solvent concentration at the swollen region, which in turn leads to a sharp solvent penetration front between the swollen region and the inner polymer core. Alternatively, solvent concentration may be fairly constant in the swollen region behind the solvent penetration front or the solvent penetration front may be sharp and advancing at a constant rate. In the latter case, diffusion distance is directly proportional to time:

\[ M_t = kt \]

Finally, there may be an induction time to Fickian diffusion, which precedes the solvent penetration front into the glassy core [192]. Fickian and Case II diffusion processes are considered as limiting types of transport whereas anomalous diffusion lies in between and is characterised by the equation

\[ M_t = kt^n \]

and

\[ \frac{1}{2} < n < 1. \]

Brazel and Peppas [187] investigated solvent and solute transport in swellable hydrophilic glassy polymers using two dimensionless parameters, the Deborah number, \( (De) \), and the swelling interface number, \( (Sw) \). The diffusional Deborah number relates net solvent motion to the rate of polymer relaxation, and is defined as:

\[ De = \frac{\lambda}{\theta} \]
where $\lambda$ is the characteristic relaxation time for the polymer when subjected to swelling stresses:

$$\lambda = \frac{\int sG(s) \, ds}{\int G(s) \, ds}$$

and $\theta$ is the characteristic penetrant diffusione time into a swelling sample, as is defined as the square of the diffusional distance divided by the diffusion coefficient of water in the polymer:

$$\theta = \frac{\delta^2}{D_s}$$

$G(s)$ is the shear relaxation modulus, $\delta$ is the thickness of the sample, and $D_s$ is the dissolution medium coefficient. Fickian diffusion is observed for $De >> 1$ or for $De << 1$. Anomalous diffusive behaviour occurs when the Deborah number is of the order of 1. Case II transport is observed when the glassy/rubbery front moves at a constant velocity. $Sw$ defines the relationship between the mobility of the diffusing solute (drug) and the penetrating dissolution medium and is given by:

$$Sw = \frac{v\delta(t)}{D_i}$$

where $v$ is the velocity of the swelling interface, $D_i$ is the concentration-independent diffusion coefficient of the drug and $\delta(t)$ is the time-dependent thickness of the rubbery, gel-like layer.

When the rate of solute transport through the solvated region is faster than the rate at which the glassy/rubbery front advances, the swelling interface number $Sw$ is much smaller than 1, and zero-order release kinetics of the drug is observed. If the swelling front advances faster than the release of the drug, the value of $Sw$ is much greater than 1 and Fickian release is observed. For values of $Sw \approx 1$, non-Fickian, non-zero order release is observed.
1.7.2. Tensile testing

Several methods have been developed to measure the bond-strength of mucoadhesive materials. Various techniques that measure the tensile force required to break the adhesive bond between a model membrane and a test polymer have been reported [183,193]. Smart et al. [153] investigated the force required to detach a test polymer from a mucus or mucus glycoprotein gel.

A variety of model mucosal surfaces have been utilised, such as Rat intestine [170], rabbit gastric mucosa [183], bovine conjunctiva [155], porcine cornea [159], ovine intestine [194], mucin-covered filter paper disks [152], cellulose dialysis tubing [159], cellulose paper impregnated with porcine gastric mucin [155].

There are number of methods for determining the force applied to the mucoadhesive joint. Smart et al. [153] developed a method for the measurement of bioadhesiveness that is similar to the Wilhelmy plate method. The apparatus consisted of a glass plate suspended from a microbalance. A 5 ml glass vial containing a mucus sample was placed in a water-bath at 20°C, and the water-bath positioned on a moveable platform. The platform was raised until the glass plate penetrated the mucus and was then left in contact with the mucus for a predetermined period of time before the platform was lowered at a rate of 1 mm/min. The maximum force recorded by the microbalance and displayed on a recorder when the plate became detached was noted. Bioadhesive polymers may be studied using this apparatus by coating the glass plate with a solution of the test material and oven drying to a constant weight.

Gurny et al. [175] developed a tensile method using an Instron tester equipped with a special cell for the determination of the adhesive bond strength. The cell was constructed using two 10 cm diameter Plexiglass discs connected through the centres by metal bars, which were attached to a tensile tester. Two cylindrical chambers enclosed the discs. The
bioadhesive material under tested was placed between the discs and held at an initial distance of 2 mm. The discs were pulled apart at an extension rate of 0.1 mm/min and the stress/strain curves were recorded.

Smart [170] assessed the mucoadhesiveness of a number of polymers using a tensile apparatus which consisted of a top pan balance beneath which was suspended a 1.5 g hooked weight. The polymer test disc was attached to the weight using a cyanoacrylate adhesive. A section of rat intestinal mucosa was mounted on a platform in isotonic phosphate buffer at 37°C to expose a 1.1 cm diameter circle of tissue. The test disc was lowered onto the mucosal surface and left for 1-2 minutes after which the platform was lowered at a rate of 1 mm/min until the disc was pulled clear of the membrane. The force at which the adhesive bond failed, the maximum detachment force, was recorded.

1.7.3. *Infrared spectroscopy*

Although a number of physical methods have been used to establish the compatibility of polymer blends, including thermal analysis and NMR spectroscopy, none of these techniques is conducive to rapid analysis of the kinetics of the interactions within the blends. FT-IR spectroscopy can be used to establish the nature and level of molecular interactions of blends and the changes in these interactions with aging [195]. In terms of IR, the compatibility of a blend may be defined by the presence of an interaction spectrum that arises when the spectrum of the blend is compared with the spectra of the two homopolymers. If the homopolymers are compatible, an interaction spectrum with frequency shifts and intensity modifications that are intrinsic to the system will be observed.

The energy range of the infrared region corresponds to that required to cause vibrational excitation of bonds within a molecule. The types of bond excitation that can
occur are stretching (higher energy) and bending (lower energy) vibrations. Absorption of certain wavelengths of infrared radiation can often be correlated with the bending and stretching of specific types of bond within a molecule. However, any single vibrational excitation cannot be considered in isolation from other parts of the molecule. Infrared spectra of organic molecules are usually complicated by bond oscillations within the whole molecule affecting absorption of the incident radiation and giving rise to overtones and harmonics. Thus, in addition to observing absorption due to individual bond vibrational excitations, absorption due to molecular vibrational excitations is also observed. Spectra recorded in solution may also show further complexity due to hydrogen bonding with solvents or the presence of dimeric or polymeric associated species.

Vibrational frequencies can be calculated using the 'ball and spring' model and applying Hooke's Law to correlate frequency with bond strength and atomic mass:

\[ v = k \sqrt{\frac{\text{bond strength}}{\text{mass}}} \]

where \( v \) is the frequency of vibration and \( k \) is the force constant of the bond, that becomes

\[ v = \frac{1}{2\pi} \sqrt{\frac{k}{m_1 m_2 \left[ \frac{1}{m_1} + \frac{1}{m_2} \right]}} \]

where \( m_1 \) and \( m_2 \) are the masses of the two constituent atoms and \( \frac{m_1 m_2}{m_1 + m_2} \) is the reduced mass of the system (\( \mu \)).

Since numerical values of \( v \) in \( s^{-1} \) are inconvenient, absorptions are usually given in 'wavenumbers':

\[ \bar{v}[cm^{-1}] = \frac{1}{\lambda[cm]} = \frac{\nu[s^{-1}]}{c[cm^{-1}]} \]

where \( c \) is the speed of light (\( 2.98 \times 10^{10} \) cm \( s^{-1} \)).
It is not usually necessary to carry out calculations using these formulae, as it is usually sufficient to use the guideline that the vibrational frequency of a bond should increase as the bond strength increases and when the reduced mass of the system decreases. Bond stretching requires more energy than bond bending, and so bond stretching absorptions require shorter wavelength (higher frequency) radiation than bond bending absorptions. Systems containing double and triple bonds require progressively higher energies for vibrational excitation.

Molecules have characteristic bending and stretching modes. A molecule of the form \( \text{XY}_2 \) (eg \( \text{CH}_2, \text{NH}_2 \)) may have a symmetric or asymmetric stretching mode. A number of bending modes are also possible and these are termed scissoring, rocking, twisting and wagging (see Fig.1.14).

![Fig. 1.13. 'Ball and spring' schematic diagram showing two types of vibrational motion in molecules](image)

![Fig. 1.14. Vibrational modes of an \( \text{XY}_2 \) molecule](image)
Some of these modes of stretching and bending, in particular symmetric stretching and scissoring, give rise to only weak IR absorption [196]. In addition, not all frequencies will give rise to absorption as they may have frequencies outside the normal infrared region or may by masked by absorption due to other, stronger frequencies.

Infrared spectra are usually recorded over the range 4000-400 cm\(^{-1}\). The radiation source is an electrically heated Nernst filament (mixed Zr, Th and Ce oxides) or a Globar filament (silicon carbide). The interferogram produced is submitted to Fourier transform and converted into a plot of absorption against wavenumber.

FT-IR spectroscopy has been used by a number of groups to investigate the formation of intermacromolecular complexes between various polymers. Ozeki et al. [197] produced interpolymer complexes of poly(ethylene oxide) and Carbopol and subjected them to FT-IR. The spectra showed that the amount of the complex formed by hydrogen bonding changed depending on the grade of Carbopol used. Lee et al. [139] prepared interpenetrating polymer network hydrogels of poly(ethylene glycol) and chitosan by UV irradiation. The use of FT-IR and Raman spectroscopy confirmed the presence of interactions between the two polymers.

1.7.4. UV-Visible spectroscopy

Organic molecules absorb radiation in discrete packets known as quanta of energy:

\[ \Delta E = hf = \frac{hc}{\lambda} \]

where \( \Delta E \) is the energy difference between the ground state and the excited state of a molecule, which is overcome by the incident radiation of frequency \( v \), \( h \) is Planck's constant, \( c \) is the speed of light and \( \lambda \) is the wavelength at which the species absorbs. The absorbed energy causes electronic transitions in the molecule, a process known as
excitation. This motion is quantized and absorption occurs only when radiation supplying exactly the right quantum of energy impinges on the compound under investigation. A molecule can undergo many different types of excitation, each of which requires its own distinctive energy \( \Delta E \). Ultraviolet (UV) and visible light are capable of moving valence shell electrons, typically from a filled bonding molecular orbital to an unfilled anti-bonding one.

The UV spectrometer has an appropriate source of electromagnetic radiation with a wavelength in the UV region (usually 200 to 800 nm). The radiation is frequently split into an incident beam and a reference beam of equal intensity. The incident beam travels through a sample tube while the latter is unperturbed. A detector at the end of the spectrometer measures the intensity of both beams and records any difference between the two. Radiation of a specific frequency, within the range of the source, passes through the sample. Wherever the sample absorbs incident light, the resulting intensity difference, relative to the reference beam, is measured by the detector and electronically relayed to the recorder to give a peak, the height of which is directly proportional to the concentration of the absorbing species.

UV spectroscopy is used to obtain the position of absorption of the species of interest, \( \lambda_{\text{max}} \) nm) which gives information on the energy \( \Delta E \) of the electronic excitation and on the chromophore responsible for the absorption. UV spectroscopy is also used to determine the molar absorptivity, also known as the molar extinction coefficient \( (\varepsilon) \). This parameter, which is a constant for a given molecule at a specific wavelength, is a measure of the ease of the transition caused by the absorption of the radiation. If a particular electronic transition can take place readily, then the light will be absorbed strongly and \( \varepsilon \) will have a high value. If the transition does not occur readily, the \( \varepsilon \) value will be low.
Substituents, which increase the extinction coefficient of the basic chromophore, are said to have a hyperchromic effect whereas those that decrease $\varepsilon$ have a hypochromic effect.

Due to the effects of superimposed vibrational and rotational transitions, absorption bands are broad; usually the maximum value of $\varepsilon$ is measured at the corresponding wavelength. For dilute mixtures of non-interacting components, absorbances are additive.

Comparison between $\varepsilon$ values for different compounds is only valid if the analyses are run with solutions at the same molarity. The relationship between the molar absorptivity, $\varepsilon$, and the concentration of the chromophore (mol L$^{-1}$) is expressed by the Beer-Lambert Law:

$$\log\left(\frac{I_0}{I}\right) = \varepsilon cl$$

where $I_0$ is the intensity of incident light, $I$ is the intensity of transmitted light, $\varepsilon$ is the molar absorptivity (units: L mol$^{-1}$ cm$^{-1}$; or, in SI, m$^2$ mol$^{-1}$), $c$ is the concentration in mol L$^{-1}$, and $l$ is the pathlength of the absorbing solution in cm.

The equation is often stated as:

$$\varepsilon = A/cl$$

where $A$ is the absorbance, $\log (I_0/I)$

UV-visible spectrometers record absorbance directly and the standard cuvette is designed to give a pathlength of 1 cm. Thus determination of $\varepsilon$ may be obtained by a simple calculation as the equation becomes:

$$\varepsilon = A/c$$

The derivation of this relationship assumes that all molecules present contribute to the absorption of the incident beam and that no absorbing molecule is in the shadow of another. For the dilute solutions usually used in UV-visible spectroscopy, this is a valid assumption [196].
1.7.5. Gel permeation chromatography

Gel permeation chromatography (GPC) has been employed in this work for the determination of the molecular weight distribution of the synthesised polymers.

Number average molecular weight ($\overline{M} _n$) is the sum of the products of the molar mass of each fraction multiplied by its mole fraction.

$$\overline{M} _n = \sum X_i M_i$$

where $X_i$ is the mole fraction of molecules of molecular mass $M_i$ – given by the ratio of the number of molecules $N_i$ with mass $M_i$ to the total number of molecules.

$$\overline{M} _n = \frac{\sum N_i M_i}{\sum N_i}$$

$\overline{M} _n$ can be determined from the colligative properties of the polymer, e.g. osmotic pressure (an affect large enough to be measurable for, in molar terms, dilute solutions).

The weight average molecular weight ($\overline{M} _w$) is defined as the sum of the products of the molar mass of each fraction multiplied by its weight fraction and can be determined by light scattering or sedimentation measurements.[154]

$$\overline{M} _w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

The polydispersity index (heterogeneity index) is the ratio of $\overline{M} _w$ and provides a measure of the breadth of the molar mass distribution (for synthetic polymers PDI increases as the molecular weight range increases). For a mono-disperse polymer, e.g. an enzyme
molecule, $\frac{M_w}{M_n} = 1.00$, but typical values range between 1.5 and 2.0 (larger and smaller values can also be obtained).

Gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC), involves a non-interactive mode of separation. The column packing is made of porous particles that contain pores of various sizes. The hydrodynamic molecular volume (size and shape) of the compounds under analysis determines the extent of their interaction with the pores. As the sample passes through the column, the molecules of larger hydrodynamic volume are unable to enter most of the pores and are eluted relatively quickly. Smaller hydrodynamic volume molecules are able to enter more pores and as a result are retained for longer [199].

The column packing is usually made of crosslinked polymers, such as styrene that is crosslinked with divinylbenzene, or 2-hydroxyethyl methacrylate that is crosslinked with ethylene dimethacrylate (pore sizes $10 - 10^5$ nm); porous glasses and silicas can also be used (pore sizes 12.5 - 100 nm). The particle sizes of the packing materials range from 5 to 10 μm [200].

1.7.6. Drug release studies

The drug release process is influenced by the physico-chemical properties of the drug and its vehicle, as well as the physiological and physical chemical properties of the biological system in which it is placed. Factors that must be considered when designing a slow release dosage form include drug concentration, aqueous solubility, molecular size, crystal form, protein binding and pKₐ. If a drug compound is homogeneously distributed throughout the matrix of an erodible tablet, the drug is said to dissolve into the polymer matrix. As the drug is released, the distance for diffusion becomes increasingly greater and
the boundary between the drug and the empty matrix recedes into the bulk matrix as the
drug is eluted.

The release of a drug from a granular matrix involves the simultaneous penetration
of the surrounding liquid, and the dissolution and leaching out of the drug through
interstitial pores. A granule is defined as a porous matrix rather than a homogeneous one.
Higuchi [201] developed an equation for the release of a drug from an ointment base,
which he later applied to the diffusion of solid drug, dispersed in homogenous and granular
matrix dosage forms:

\[ Q = \left[ D(2A - C_s)C_s t \right]^{1/2} \]

where \( Q \) is the amount of drug depleted per unit area, \( D \) is the diffusion coefficient of the
drug, \( A \) is the total concentration of drug in the homogenous matrix, \( C_s \) is the solubility of
the drug in the polymer matrix and \( t \) is the time at which the solution of the equation is
true.

In a porous matrix, a second form of the Higuchi equation is used, which takes into
account the volume and length of the opening in the matrix:

\[ Q = \left[ \frac{D\varepsilon}{\tau} (2A - \varepsilon C_s C_s t) \right]^{1/2} \]

where porosity, \( \varepsilon \), is the fraction of the matrix that exists as pores into which the
surrounding fluid can penetrate and tortuosity, \( \tau \), accounts for the increase in the path
length of diffusion due to branching and bending of the pores. Tortuosity tends to reduce
the amount of drug released in a given time. A straight channel has a tortuosity of one,
while the tortuosity of a channel going through a bead bed of uniformly sized beads lies
between 2 and 3.

The results obtained from \textit{in vitro} drug release experiments may be fitted to a
number of mathematical models to determine the release characteristics. These
characteristics can then be used to assess the usefulness of the formulation as a controlled release system.

Typically, zero-order drug release systems consist of a drug reservoir, containing a homogeneously suspended drug and enclosed by a rate-limiting barrier. Such systems maintain a constant surface area and activity gradient.

In cases of first-order release, the drug activity within the reservoir declines exponentially and the rate of drug release is proportional to the residual activity. The reservoir typically contains only dissolved drug and a plot of the logarithm of residual drug against time is linear with the slope being equal to the rate constant:

\[ \ln(1 - \frac{M_t}{M_\infty}) = -kt \]

where \( \frac{M_t}{M_\infty} \) is the fractional drug release at time \( t \) (\( M_t \) is the amount of drug released at time \( t \), and \( M_\infty \) is the initial amount of drug in a tablet), \( k \) is a constant incorporating the properties of the macromolecular polymeric systems and of the drug.

![Diagram of zero-order and first-order drug release](image)

**Fig. 1.15.** Zero-order and first-order drug release from polymer matrices
The Hixson and Crowell cube-root equation

Hixson and Crowell [202] developed a model to describe drug release from systems that showed dissolution-rate limitation. This model may be applied to any such system that does not change in shape as drug release proceeds. The following equation is used to describe this process:

\[(1 - \frac{M}{M_\infty})^{\frac{1}{3}} = -kt\]

where \(M/M_\infty\) is the fractional drug release at time \(t\) (\(M_t\) is the amount of drug released at time \(t\), and \(M_\infty\) is the initial amount of drug in a tablet), \(k\) is a constant incorporating the properties of the macromolecular polymeric systems and of the drug.

Higuchi square root of time model

Higuchi [203] showed that drug release into a sink system consisting of drug dispersed within a diffusion rate-limiting planar matrix could be modelled, at steady state, using the following equation:

\[(\frac{M}{M_\infty})^2 = kt\]

where \(M/M_\infty\) is the fractional drug release at time \(t\) (\(M_t\) is the amount of drug released at time \(t\), and \(M_\infty\) is the initial amount of drug in a tablet), \(k\) is a constant incorporating the properties of the polymeric system and of the drug.

Roseman and Higuchi equation for cylindrical matrices

Roseman and Higuchi [204] showed that the drug release profile for a cylindrical matrix containing a suspended drug, may be modelled by the following equation:

\[\frac{M_t}{M_\infty} + [1 - \frac{M_t}{M_\infty}] \ln [1 - \frac{M_t}{M_\infty}] = \frac{4CsD}{Aa^2} t\]
where $M_t/M_\infty$ is the fractional drug release at time $t$ ($M_t$ is the amount of drug released at time $t$, and $M_\infty$ is the initial amount of drug in a tablet), $a_o$ is the radius of the cylindrical matrix, $A$ is the drug loading, $C_s$ is the drug solubility in the polymer, and $D$ is the drug diffusivity in the polymer phase. According to Roseman and Higuchi's assumptions, this equation is more applicable to cylindrical matrix systems when the drug loading is much higher than solubility of the drug in the polymer.

The correlation between \textit{in vitro} and \textit{in vivo} drug release is usually determined experimentally. \textit{In vitro} release profiles of pharmaceutical preparations and subsequent fitting of the resulting data to mathematical models are useful, particularly if the preparations appear to be identical but are suspected to behave differently from one another \textit{in vivo}.

1.8. Project Aims

Efficient drug delivery to the eye still represents a major challenge, despite the considerable research effort dedicated to it in the last decade. Previous studies have attempted either to increase the bioavailability of ophthalmic drugs by making use of methods that aim either, to improve the corneal penetration of the active and to delay its elimination from the eye, or, to to achieve controlled release of the drug by using various types of ophthalmic inserts.

This project is directed towards the preparation and evaluation of novel polymeric vehicles that can effect the sustained (controlled) release of ocular drugs. It aims to combine recent advances in mucoadhesion and polymer-based drug delivery for the purpose of designing a new generation of aqueous ophthalmic vehicles. Previous work at the University of Portsmouth has established the great promise of polycarbophil and
chitosan as materials that can adsorb from solution onto unfixed human buccal epithelial cells [182], and it is envisaged that, based on these types of polymeric materials, ocular wafers or a ‘nanoparticulate’ formulation can be developed. Within the programme of work, several objectives are outlined:

i) Synthesis and characterisation of novel mucoadhesive hydrogels that are based on synthetic monomers and naturally-derived polymeric units, such as N-vinylpyrrolidone, (meth)acrylic acid, N-isopropylacrylamide or 2-hydroxyethyl methacrylate, and chitosan with different molecular weights;

ii) Formulation of these hydrogels either as ophthalmic inserts or as nanosuspensions;

iii) Evaluation of their swelling properties, adhesion behaviour and in vitro drug release characteristics, using a range of common ophthalmic drugs; and

iv) In vivo evaluation of the most promising formulations.

Polymers that are known to hydrate at a rapid rate, but to a limited extent, will be selected for mucoadhesion testing; rapid gelation is required for patient comfort and to allow adhesion and drug release to commence quickly, whereas limited swelling is required to prevent break-up and dissolution of the gel and to provide prolonged retention and long-lasting drug delivery. Formulations will be based mainly on polymers with regulatory approval, such as polysaccharides (chitosan), polyacrylates (acrylic acid, methacrylic acid) and poly(vinylpyrrolidone)s.

Model therapeutic agents including pilocarpine, chloramphenicol, atropine, and norfloxacin will be incorporated within the polymer matrix; aqueous nanoparticulate suspension will be prepared and tested both in vitro and in vivo.

An in vitro model, which utilises a radiolabelled marker, could be also employed in order to simulate the penetration of the particulate formulations through the corneal tissue.
1.9. References


Chapter 2 - Poly(vinylpyrrolidone-co-[meth] acrylic acid) inserts for ocular drug delivery

2.1. Introduction

The efficient ophthalmic delivery of drugs (β-blockers, cholinergic or antibacterial agents) continues to be challenged by the intrinsic physiology of the eye. The removal mechanisms that operate at the site of action (rapid tear turnover, blinking) and the low corneal permeability act co-operatively to suppress the effectiveness of ophthalmic formulations and to limit drug bioavailability to less than 5% [1]. Pulsed drug entry following topical administration and a very low efficiency for drug delivery to the posterior segment of ocular tissue, represent further therapeutic deficiencies [2,3]. Undesirable side effects, induced by systemic exposure through drainage via the nasolachrymal duct, are also an important issue of concern.

Attempts have been made to improve the corneal penetration of drug molecules and delay their elimination from the eye. These include the use of gels, viscosity-enhancing agents, ointments, penetration enhancers, phase transition systems, cyclodextrins, vesicular systems, prodrugs, microemulsions or nanosized cationic gels and devices (e.g. inserts, collagen shields, ocular films or disposable contact lenses) [2-7]. However, only ocular inserts [8] and nano/micro-particles [9,10] show significant promise in terms of effective, controlled and sustained drug release [2,4].

As potential drug carriers, polymers based on 1-vinylpyrrolidin-2-one (VP), acrylic acid (AA) and methacrylic acid (MA) are well known for their biocompatibility [11-13]. VP-based polymers, used in contact lenses for many years, are promising drug carriers [14-16], while crosslinked polyacrylic acid is known to have excellent mucoadhesive properties and to enhance ocular bioavailability [17-19]. Blends of poly(1-vinylpyrrolidin-
2-one) (PVP) and polyacrylic acid (PAA) have been reported to exhibit low viscosity characteristics and a high mucoadhesion index [20], and recent interpolymer-complexation studies have indicated that these blends are very promising candidate materials for ophthalmic drug delivery systems [21]. Their advantages over homopolymers include prolonged association with the cornea (hence an increased bioavailability of the loaded drug), controlled release, transparency and comfort [22-24]. Although crosslinked materials based on VP and AA (or MA) copolymers have been considered as vehicles for drug delivery systems [25], there are no detailed studies of their potential as ophthalmic carriers.

This work describes the preparation and characterisation of ocular inserts based on VP/AA[MA] copolymers, and present the results of in vitro and in vivo investigations into their potential to act as vehicles for drug delivery to the eye.

2.2. Materials and methods

2.2.1 Synthesis

VP (Sigma-Aldrich) was further purified by vacuum distillation; AA and MA were passed through an inhibitor removal column (HQ, Sigma-Aldrich). Solvents (Fischer), the polymerisation initiator 4,4'-azobis(4-cyanovaleric acid) (ACVA, Sigma-Aldrich) and the model drugs (Fluka) for controlled release studies were used as received. Phosphate buffer solutions (PBS, pH = 7.4, ionic strength 0.497 M) were prepared from sodium monohydrogen phosphate, sodium dihydrogen phosphate and NaCl [26].

A parallel synthesis kit (Büchi Syncor) was used for the preparation of the polymeric networks. Aqueous solutions of co-monomers (see Table 2.1 for feed ratios) were mixed with 4,4'-azobis(4-cyanovaleric acid) (1 %w/v) and stirred under N₂ for 8 h at 80 °C). After removal of water, the polymers were freeze-dried, finely ground, purified by
Soxhlet extraction (THF, 6h), and dried (vacuum oven, 40°C; see Table 2.1 for yields). Infrared spectra (KBr discs, resolution 4 cm⁻¹) were recorded (Mattson RS-1). Wafers were prepared by embedding the powdered drug uniformly in the hydrogel matrix (2 %w/w; 4 mg of polymer in 200 mg of dry KBr) followed by compression (2 tonne, for 1 min).

2.2.2. Swelling and adhesion measurements

The swelling properties at physiological pH (7.4) were determined volumetrically with at least three measurements for each value of the equilibrium degree of swelling, DS_{eq} (%):

\[ DS_{eq} = \frac{v_{eq} - v_0}{v_0} \times 100 \]

where \( v_0 \) and \( v_{eq} \) are respectively the initial and equilibrium sample volumes. The polymer sample (30 mg) and 0.5 ml of PBS buffer were added to a 1 ml microsyringe, and the increase in the volume of the sample was noted at preset time intervals (5 min × 6, then 30 min to equilibrium).

Adhesion measurements were performed on polymer wafers (Ø 8 mm; prepared from 30 mg of polymer, by applying 2 tonne for 1 min), in PBS (pH=7.4), using hydrated dialysis tubing membrane (cellulose, Visking DTV14000) as the test surface. A Stable Microsystem TAXT Plus Analyzer was used for this purpose; the maximum detachment force (MDF) and the corresponding work of adhesion (TWA) were determined from the recorded Force-Distance curves (see image for an example) using the standard TAXT Plus Analyzer software.

Carbopol C971 (BF Goodrich) was employed as positive control. The test
parameters used for the adhesion test are presented below:

<table>
<thead>
<tr>
<th>Pre-test speed</th>
<th>30 mm / min</th>
<th>Applied force</th>
<th>20 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test speed</td>
<td>1 mm / min</td>
<td>Return distance</td>
<td>8 mm</td>
</tr>
<tr>
<td>Post test speed</td>
<td>1 mm / min</td>
<td>Contact time</td>
<td>2 min</td>
</tr>
</tbody>
</table>

2.2.3. **Drug release in vitro**

The controlled release experiments were performed on polymeric wafers (Ø 8 mm) prepared by compression (2 tonne, 1 min) of a physical mixture of 50 mg of polymer and: 5, 10, or 20 mg of either pilocarpine hydrochloride or chloramphenicol. Thus, wafers with different drug loading were obtained (9.0 %, 16.7 %, and 28.6 %, respectively; w/w). *In vitro* release profiles under sink conditions (phosphate buffer, pH 7.4; 37 °C) were obtained spectrophotometrically using a dissolution apparatus (Copley DT-70, Erweka Instruments: 500 ml; U.S.P. II apparatus; 25 rpm) connected to a spectrometer (UV-300 Spectronic Unicam; 215 nm and 282 nm for pilocarpine and chloramphenicol, respectively) via an automatic flow-through sampling system. At the beginning of each experiment, wafers were introduced (dropped in) at the bottom of the dissolution baths, then the drug release was followed spectrophotometrically. Calibration was performed using standard solutions of drug (0.001 - 0.02 %w/w) in phosphate buffer. Blank experiments, using polymer-only tablets, confirmed that the hydrogels did not absorb significantly in the UV.

2.2.4. **Drug release in vivo**

Ocular inserts/wafers (Ø 6 mm) were prepared by the compression (2 tonne, 1 min) of a physical mixture comprising of polymer (10 mg) and pilocarpine hydrochloride (2.5 mg or 1.25 mg); inserts with different drug loading were obtained (20 %w, and 11.1 %w, respectively). Inserts (Ø 6 mm) having the same drug loading (20 % weight), but
containing a higher dose of drug, were prepared from the polymer under study (20 mg) and pilocarpine hydrochloride (5 mg), using the same method.

The induced miotic effect in male, albino New Zealand rabbits (4.0 - 4.5 kg) was assessed, until disappearance, by timed measurements of the pupillary diameter. Procedures were approved by the Ethical Review Committee at the University of Portsmouth, and all studies were performed in accordance with Home Office regulations for the care and use of laboratory animals.

Each insert was administered into the lower cul-de-sac of the left eye, while 50 μl of pilocarpine solution containing 5 mg, 2.5 mg, or 1.25 mg of pilocarpine hydrochloride were instilled into the lower cul-de-sac of the right eye. In separate experiments, empty wafers (without loaded drug) provided the blank controls.

2.3 Results and discussion

2.3.1 Yields and characterisation of copolymers

For the free-radical copolymerisation of vinyl 2-pyrrolidone (VP) with acrylic acid (AA), or methacrylic acid (MA), weight ratios of VP:AA (or VP:MA) between 1:0 and 1:1 (w/w) were used (see Table 2.1). The concentration of N,N'-methylenebisacrylamide (MBA), employed as a crosslinker, was varied over a very large range, namely between 0 and 23 %w/w. Generally, better yields were obtained as the amount of crosslinker was increased (Table 2.1).

The composition of the purified materials was examined using the C=O bands of the corresponding VP (~1650 cm⁻¹) and AA [MA] (~1720 cm⁻¹) units. The amide II band (1535 cm⁻¹, N-H bending) was used to assess the MBA ratio in the network: its displacement, from 1548 cm⁻¹ in pure MBA, indicated that the crosslinker influences the extent of the hydrogen-bonded interactions that operate within the network (Fig.2.1b).
Table 2.1. Monomers and crosslinker feed ratios (weight ratio), crosslinker concentration (%w), and polymer yields.

<table>
<thead>
<tr>
<th>Polymer code</th>
<th>VP</th>
<th>AA</th>
<th>MA</th>
<th>MBA</th>
<th>MBA (%w)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp(VP/AA)M_1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.004</td>
<td>0.2</td>
<td>66</td>
</tr>
<tr>
<td>cp(VP/AA)M_2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.04</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>cp(VP/AA)M_3</td>
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<td>1</td>
<td>0</td>
<td>0.2</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>cp(VP/AA)M_4</td>
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<td>1</td>
<td>0</td>
<td>0.4</td>
<td>16</td>
<td>95</td>
</tr>
<tr>
<td>cp(VP/AA)M_5</td>
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<td>1</td>
<td>0</td>
<td>0.6</td>
<td>23</td>
<td>95</td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
<td>0.004</td>
<td>0.2</td>
<td>59</td>
</tr>
<tr>
<td>cp(VP/AA)M_7</td>
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<td>0</td>
<td>1</td>
<td>0.04</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>cp(VP/AA)M_8</td>
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<td>0</td>
<td>1</td>
<td>0.2</td>
<td>9</td>
<td>81</td>
</tr>
<tr>
<td>cp(VP/AA)M_9</td>
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<td>0</td>
<td>1</td>
<td>0.4</td>
<td>16</td>
<td>71</td>
</tr>
<tr>
<td>cp(VP/MA)M_10</td>
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<td>0</td>
<td>1</td>
<td>0.6</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>cp(VP/AA)M_11</td>
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<td>0.25</td>
<td>0</td>
<td>0.0025</td>
<td>0.2</td>
<td>64</td>
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<tr>
<td>cp(VP/AA)M_12</td>
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<td>0.25</td>
<td>0</td>
<td>0.025</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>cp(VP/AA)M_13</td>
<td>1</td>
<td>0.25</td>
<td>0</td>
<td>0.125</td>
<td>9</td>
<td>62</td>
</tr>
<tr>
<td>cp(VP/AA)M_14</td>
<td>1</td>
<td>0.25</td>
<td>0</td>
<td>0.25</td>
<td>16</td>
<td>95</td>
</tr>
<tr>
<td>cp(VP/AA)M_15</td>
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<td>0.25</td>
<td>0</td>
<td>0.375</td>
<td>23</td>
<td>88</td>
</tr>
<tr>
<td>cp(VP/MA)M_16</td>
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<td>0</td>
<td>0.25</td>
<td>0.0025</td>
<td>0.2</td>
<td>24</td>
</tr>
<tr>
<td>cp(VP/MA)M_17</td>
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<td>0</td>
<td>0.25</td>
<td>0.025</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>cp(VP/MA)M_18</td>
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<td>0</td>
<td>0.25</td>
<td>0.125</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>cp(VP/MA)M_19</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
<td>0.25</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>cp(VP/MA)M_20</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
<td>0.375</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>cp(VP/AA)M_21</td>
<td>1</td>
<td>0.05</td>
<td>0</td>
<td>0.002</td>
<td>0.2</td>
<td>25</td>
</tr>
<tr>
<td>cp(VP/AA)M_22</td>
<td>1</td>
<td>0.05</td>
<td>0</td>
<td>0.02</td>
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<td>40</td>
</tr>
<tr>
<td>cp(VP/AA)M_23</td>
<td>1</td>
<td>0.05</td>
<td>0</td>
<td>0.1</td>
<td>9</td>
<td>63</td>
</tr>
<tr>
<td>cp(VP/AA)M_24</td>
<td>1</td>
<td>0.05</td>
<td>0</td>
<td>0.21</td>
<td>16</td>
<td>90</td>
</tr>
<tr>
<td>cp(VP/AA)M_25</td>
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<td>0.05</td>
<td>0</td>
<td>0.315</td>
<td>23</td>
<td>96</td>
</tr>
<tr>
<td>cp(VP/MA)M_26</td>
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<td>0</td>
<td>0.05</td>
<td>0.002</td>
<td>0.2</td>
<td>35</td>
</tr>
<tr>
<td>cp(VP/MA)M_27</td>
<td>1</td>
<td>0</td>
<td>0.05</td>
<td>0.02</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>cp(VP/MA)M_28</td>
<td>1</td>
<td>0</td>
<td>0.05</td>
<td>0.1</td>
<td>9</td>
<td>61</td>
</tr>
<tr>
<td>cp(VP/MA)M_29</td>
<td>1</td>
<td>0</td>
<td>0.05</td>
<td>0.21</td>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>cp(VP/MA)M_30</td>
<td>1</td>
<td>0</td>
<td>0.05</td>
<td>0.315</td>
<td>23</td>
<td>97</td>
</tr>
<tr>
<td>p(VP)M_31</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.002</td>
<td>0.2</td>
<td>61</td>
</tr>
<tr>
<td>p(VP)M_32</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.02</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>p(VP)M_33</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>p(VP)M_34</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>16</td>
<td>88</td>
</tr>
<tr>
<td>p(VP)M_35</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>
Fig. 2.1. Selected FTIR spectra: a) Poly(vinylpyrrolidone) with increasing crosslinking density (from bottom upwards: 0.2; 2; 9; 16; 23 %, feed content); b) Poly (vinylpyrrolidone-co-acrylic acid) copolymers with increasing AA ratio (from bottom upwards), crosslinked with MBA (23 %, weight, feed content); c) Poly(vinylpyrrolidone-co-acrylic acid) copolymers with a weight ratio VP/AA of 1/0.05 and different crosslinking density (from bottom upwards: 0.2; 2; 9; 16; 23 % MBA, feed content). Codes as in Table 2.1.
The spectroscopic data revealed that MA copolymers possess higher MA to VP ratios relative to the feed composition whereas, in most cases, the opposite effect was observed with AA-containing copolymers. This is explained in terms of the reactivity order, MA > VP > AA, and confirms the findings of Chapiro [27], who has also reported that methacrylic acid is more reactive than acrylic acid in its copolymerisation with vinylpyrrolidone.

The incorporation of AA[MA] co-monomer into the VP network has a major effect upon the hydrogen-bonded associations (see Fig.2.1a and 2.1c), as the competitive COOH···O=C<(PVP) interactions displace sorbed water molecules and reduce the intensity of the corresponding broad band (3600-3400 cm\(^{-1}\)), while a lower wavelength band (characteristic of strongly associated O-H bonds) appears at around 3200 cm\(^{-1}\). Notably, this effect is more pronounced at higher crosslinking densities (Fig.2.1c), possibly due to steric constraints that limit the presence of sorbed water molecules within the network. The strong absorption in the region 3600-3400 cm\(^{-1}\) highlights the hygroscopic nature of the material; water is readily sorbed by the polymer even after a very short exposure to atmospheric conditions [38-40]. The weak shoulder at around 3200 cm\(^{-1}\) is most probably due to hydrogen bonded interactions involving the N-H group (Fig. 2.1a).

For the same amount of crosslinker, increasing the relative proportion of AA[MA] results in an increase in the number of free carboxylic acid groups – presumably their ability to interact with the carbonyllic VP groups (or to self-associate as head-to-head dimers) is dictated by steric constraints within the network (see Fig.2.1b). Both free and associated >C=O groups (respectively, 1721 cm\(^{-1}\) and 1712 cm\(^{-1}\) for AA[MA] units, 1655 cm\(^{-1}\) and 1635 cm\(^{-1}\) for VP groups) were observed for all polymers, but the bands were too broad for quantitative analysis.
2.3.2. Swelling and adhesion

The effects of the hydrogels’ network structure and composition upon their swelling properties were investigated by volumetric swelling measurements, following the method described in section 2.2.2.

The equilibrium and the dynamic swelling behaviour of the synthesised materials are highly dependent upon their chemical composition. This is consistent with literature reports on methacrylate polymers in which increasing the percentage of crosslinker reduces the rate and degree of swelling and changes the water transport mechanism from Fickian diffusion to anomalous transport [28].

Under controlled conditions (37.5 °C, pH 7.4), the synthesised materials displayed a broad range of swelling behaviour. The VP/AA copolymers exhibited greater degrees of swelling and more rapid swelling rates than their VP/MA congeners; equilibration times were generally longer for the VP/AA copolymers. For both classes of copolymer, the equilibrium degree of swelling at \( DS_{eq} \) was at its maximum for materials containing 9 \( \% \) w/w of crosslinker (Fig. 2.2).

![Fig. 2.2. Comparison of the equilibrium degree of swelling, \( DS_{eq} \), between selected AA- and MA- containing hydrogels (details in Table 2.1); standard deviations are < 2 \%, \( n_{exp} = 5 \).](image)
Effect of crosslinking density and proportion of acrylic acid on the swelling behaviour of copolymers

Fig. 2.3. Swelling degree at equilibrium (DSeq) as a function of both hydrogel AA content and crosslinking density; different colours indicate the % increase in volume due to swelling.

Tablets prepared from highly crosslinked materials were more difficult to press, and had a greater tendency to disintegrate during swelling experiments. Increasing the proportion of AA/MA gave a maximum degree of swelling at equilibrium at 10-15 %w/w (Fig 2.3) beyond which the degree of swelling at equilibrium decreased. This contrasts with the progressive increases in degree of swelling and water sorption rate (with equilibration time and solvent transport mechanism remaining unchanged) that normally result from increasing the proportion of ionic co-monomers [28].

The adhesion behaviour of the synthesised poly(vinylpyrrolidone-co-[meth]acrylic acid)s was studied using a Stable Microsystem Texture Analyzer, as described in section 2.2.2. Our adhesion measurements on polymer wafers allowed the determination of the maximum detachment force (MDF) and the corresponding work of adhesion (TWA). Crosslinked polyacrylic acids (e.g. Carbopols) are known to exhibit excellent mucoadhesive properties and to enhance the bioavailability of some drugs [17]; the bioadhesive behaviour...
of Carbopols is optimal in acidic media (pH < 5), due to H-bonding between its undissociated COOH groups and mucin [29-31].

Table 2.2. Variations in the maximum detachment force (MDF) and the total work of adhesion (TWA) with crosslinker density, for the copolymers studied (SD = standard deviation; nexp = 6; means tabulated).

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Crosslinker (MBA) density (%w/w)</th>
<th>MDFav (mN)</th>
<th>SD (mN)</th>
<th>TWAav (μJ)</th>
<th>SD (μJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp(VP/AA)M 1</td>
<td>0.2</td>
<td>94</td>
<td>49</td>
<td>9.0</td>
<td>1.6</td>
</tr>
<tr>
<td>cp(VP/AA)M 2</td>
<td>2</td>
<td>38</td>
<td>10</td>
<td>8.3</td>
<td>2.2</td>
</tr>
<tr>
<td>cp(VP/AA)M 3</td>
<td>9</td>
<td>36</td>
<td>22</td>
<td>15.5</td>
<td>11.5</td>
</tr>
<tr>
<td>cp(VP/AA)M 4</td>
<td>16</td>
<td>51</td>
<td>19</td>
<td>21.7</td>
<td>11.1</td>
</tr>
<tr>
<td>cp(VP/AA)M 5</td>
<td>23</td>
<td>116</td>
<td>14</td>
<td>9.8</td>
<td>1.9</td>
</tr>
<tr>
<td>cp(VP/MA)M 6</td>
<td>0.2</td>
<td>135</td>
<td>13</td>
<td>7.8</td>
<td>0.8</td>
</tr>
<tr>
<td>cp(VP/MA)M 7</td>
<td>2</td>
<td>15</td>
<td>1</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>cp(VP/MA)M 8</td>
<td>9</td>
<td>13</td>
<td>3</td>
<td>10.0</td>
<td>3.5</td>
</tr>
<tr>
<td>cp(VP/MA)M 9</td>
<td>16</td>
<td>53</td>
<td>7</td>
<td>6.0</td>
<td>1.6</td>
</tr>
<tr>
<td>cp(VP/MA)M 10</td>
<td>23</td>
<td>63</td>
<td>14</td>
<td>25.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Both copolymer-network and PVP wafers exhibited maximum adhesion at low crosslinking densities (Table 2.2, MBA ratio 0.2-2% w), indicating that the incorporation of AA[MA] moieties into the VP network suppresses its bioadhesive properties progressively as the degree of crosslinking in the materials is reduced (Table 2.2). This effect can probably be explained in terms of the relative contributions of the strong interpolymer hydrogen bonding [COOH···O=C<PVP)] and the much weaker polymer-substrate interactions under the various crosslinking regimes. The findings are consistent with Tan’s observation [23] that, in Carbopol-PVP complexes, the adhesion properties are reflective of Carbopol content. Interestingly, Tan et al. [23] also found that the adhesion strength of the copolymers was lower than that of corresponding physical mixtures of the constituent polymers. Also, Chun et al. [22] have reported that PVP/PAA complexes are more bioadhesive than pure Carbopol.
2.3.3. Drug release studies

Since prolonged retention, minimal discomfort and disruption of vision are critical requirements for ocular inserts, only the polymers containing 0.2 and 2.0 %w/w MBA, i.e. those that showed at least minimal adhesive properties that were coupled with slow and limited swelling, were selected for further studies. Pilocarpine (cholinergic agonist, a common antiglaucoma drug) and chloramphenicol (a broad-spectrum antibacterial agent) were selected as model drugs for the in vitro studies (Fig. 2.4).

Fig. 2.4. Drug structures: a) pilocarpine hydrochloride; b) chloramphenicol.

Copolymers were loaded with model drugs by physical mixing (grinding the two powders together) and pressing into a tablet, and the release of the active was monitored spectrophotometrically over time using a dissolution bath connected to a UV spectrometer via an automatic flow-trough sampling system, as described in section 2.2.3. In some cases, gradual tablet disintegration has been observed over the course of the experiment.
Fig. 2.5. Chloramphenicol (10 mg in 50 mg of copolymer) in vitro release profiles for materials (Table 2.1) with different VP/AA ratios, and with different crosslinking densities (PBS, pH = 7.4; 37 °C; n_exp = 6; means represented; standard errors were < 10 %).

Overall, differences in release rates between systems that contain PVP only (i.e. p(VP)M_31 to 35) and copolymers with AA and MA (i.e. materials numbered _1 to _30, Table 2.1) were less pronounced with pilocarpine than with chloramphenicol. The results suggest that the more hydrophilic the delivery vehicle the less suitable it is for applications that demand the immobilisation of water-soluble, low molecular weight drugs. Nonetheless, highly hydrophilic gels may be useful for the release of active agents that are sparingly soluble in water (e.g. hydrocortisone-acetate), or for that of water-soluble macromolecules (enzymes, proteins, antigens) that can be immobilized in the hydrophilic matrix by physical entanglement [32].
Fig. 2.6. Pilocarpine in vitro release profiles for materials (Table 2.1) with different VP/AA ratios, and with different crosslinking densities; (10 mg pilocarpine hydrochloride in 50 mg of polymer; PBS, pH=7.4; 37 °C; n_exp = 6; means represented; standard errors were < 10 %).

The materials with intermediate crosslinking densities (2 %w/w MBA) released the drug more rapidly than those with 0.2 %w/w MBA (Figs. 2.5 & 2.6). For these materials there appears to be an optimum AA[MA] content for slow release. Statistical treatment of the results (ANOVA, two-factors with replication; 5 replicates; p < 0.05) indicated that there is a significant difference between the materials with 2 %w/w crosslinking densities and materials with 0.2 %w/w crosslinking densities.
Fig. 2.7. Release profiles for pilocarpine hydrochloride and chloramphenicol from AA and MA based copolymers (a - cp(VP/MA)M_6, and b - cp(VP/AA)M_21); (10 mg model drug in 50 mg of polymer; PBS, pH=7.4; 37 °C; n_exp = 6; means represented; standard errors were < 10 %).

For both types of materials tested, the initial release of chloramphenicol was slower compared to that of pilocarpine (Fig. 2.7). Up to ca. 50 % of drug released, the release rate
was approximately constant for chloramphenicol, whereas for pilocarpine it decreased progressively.

The observed differences in the release profiles of the two drugs (Fig. 2.7) can be, at least partially, explained in terms of their relative solubility in water; the chloramphenicol structure allows for stronger hydrogen-bonded interactions between the polymer matrix and the active (Fig. 2.4). It is worth noting that uncrosslinked copolymeric VP/AA hydrogels are more hydrophilic in neutral and alkaline media than in acidic environments [25].

To investigate the differences in release rates characterising the two actives, drug release exponents \((n)\) were determined using the well-known expression, used for example by Korsmeyer et al.[33]:

\[
\frac{M_t}{M_\infty} = kt^n
\]

where \(\frac{M_t}{M_\infty}\) is the fraction of drug released, \(t\) is the release time and \(k\) is a constant incorporating the structural and geometrical characteristics of the release device. From here, \(\log(\text{gradient}) = (n-1) \cdot \log t + \text{const}\), hence ‘constant rate’ corresponds with “\(n = 1\)”. The values of \(n\) were derived from the linear regression slopes of the release profiles (0–80 %) using the above expression. Release exponents (Table 2.3) for chloramphenicol-based systems were significantly greater than for those incorporating pilocarpine; if values for the latter active were interpreted as indicative of a release mechanism that ranges from Fickian \((n = 0.5)\) to anomalous diffusion, a Super Case II transport mechanism [34] would appear to operate for most chloramphenicol-based systems.

However, the basis of the Korsmeyer equation takes no account of the complex mechanism - involving parallel swelling, diffusion and erosion - that is integral to the process of drug release from hydrophilic matrices [35-37]. With only a few exceptions, release rates were higher for pilocarpine (Table 2.3); consistent with its inability to act as an H-bond donor to the polymer matrix.
Table 2.3. Comparison of release exponents \((n)\) and specific release rates \((k)\) with copolymer composition \((R^2 = \text{correlation coefficient})\).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drug</th>
<th>chloramphenicol</th>
<th>pilocarpine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n)</td>
<td>(k / h^n)</td>
</tr>
<tr>
<td>~p(VP)M 12</td>
<td>chloramphenicol</td>
<td>1.52</td>
<td>6.26</td>
</tr>
<tr>
<td>cp(VP/AA)M 22</td>
<td>chloramphenicol</td>
<td>1.29</td>
<td>1.46</td>
</tr>
<tr>
<td>p(VP)M 32</td>
<td>chloramphenicol</td>
<td>0.97</td>
<td>0.423</td>
</tr>
<tr>
<td>cp(VP/AA)M 11</td>
<td>chloramphenicol</td>
<td>1.29</td>
<td>1.73</td>
</tr>
<tr>
<td>cp(VP/AA)M 21</td>
<td>chloramphenicol</td>
<td>1.04</td>
<td>0.115</td>
</tr>
<tr>
<td>p(VP)M 31</td>
<td>chloramphenicol</td>
<td>0.90</td>
<td>0.885</td>
</tr>
<tr>
<td>cp(VP/MA)M 6</td>
<td>chloramphenicol</td>
<td>0.86</td>
<td>0.300</td>
</tr>
<tr>
<td>cp(VP/MA)M 26</td>
<td>chloramphenicol</td>
<td>0.92</td>
<td>0.402</td>
</tr>
<tr>
<td>cp(VP/MA)M 7</td>
<td>chloramphenicol</td>
<td>1.31</td>
<td>1.11</td>
</tr>
<tr>
<td>cp(VP/MA)M 27</td>
<td>chloramphenicol</td>
<td>1.53</td>
<td>1.32</td>
</tr>
</tbody>
</table>

The significant influences on release rates of both drug-polymer interactions and matrix hydrophobicity have been shown: while increasing the proportion of acrylic to VP co-monomer produces copolymers that exhibit faster release rates, its replacement by methacrylic acid (more hydrophobic) has the opposite effect (as indicated by values of \(k\); Table 2.3).

2.3.4. In vivo experiments

The New Zealand albino rabbit model was employed for the in vivo studies (two animals; rest period between experiments 48 h). As a positive control, an aqueous solution of the drug was administered dropwise; drug-free wafers (blank controls) confirmed that the pure hydrogels had no contribution to the observed miotic effect. The variation in pupillary diameter was monitored over time using a standard pupillary diameter gauge. In all observations, a sharp initial effect was followed by a slow return towards the original level (Fig. 2.8).
Fig. 2.8. Pupillary diameter profile (rabbit) following the administration of 2.5 mg pilocarpine hydrochloride formulated in cp(VP/MA)M_6 and p(VP)M_32 (10 mg); control = aqueous solution containing the same amount of drug as sample; bars represent standard errors, n_{exp} = 6.

The inserts induced a more pronounced miotic effect (decrease in pupillary diameter) than the eyedrop control, and the period of significant activity increased by up to 110%. The
results reveal that materials with reduced hydrophilicity, which have a limited swelling capacity, yield more desirable activity profiles (Fig. 2.9).

Fig. 2.9. Pilocarpine-induced profiles of miotic effect (change in pupillary diameter) obtained in vivo for a series of materials with different composition and various degrees of drug loading (11% - prepared from 1.25 mg pilocarpine and 10 mg copolymer; 20% - prepared from 2.5 mg pilocarpine and 10 mg copolymer, or 5 mg pilocarpine and 20 mg copolymer) compared with the corresponding aqueous solution controls (C/1.25, C/2.5, and C/5 – containing 1.25 mg, 2.5 mg, and 5 mg pilocarpine, respectively); standard errors were < 15%; n_exp = 6.
Statistical treatment of the results (ANOVA, two-factors with replication; 6 replicates; \( p < 0.05 \)) indicated a highly significant difference (in terms of the induced miotic effect) between some of the materials \([\text{cp}(\text{VP/MA})M_6; \text{p}(\text{VP})M_{32}]\) and control solution, and a highly significant interaction (in terms of the induced miotic effect) between the same materials \([\text{cp}(\text{VP/MA})M_6; \text{p}(\text{VP})M_{32}]\) and time.

The concentration of methacrylic acid (more hydrophobic than acrylic acid) and the degree of crosslinking seem to be the key factors that control pilocarpine release. Loading with greater amounts of pilocarpine produced only a slight increase in the duration of the pilocarpine-induced miotic effect, possibly due to the limited drug loading capacity of the materials.

### 2.4. Conclusions

A number of hydrogel polymeric networks were prepared by the free radical copolymerisation of vinylpyrrolidone with methacrylic or acrylic acids, in the presence of \( N,N' \)-methylenebisacrylamide as a crosslinking agent. Dependent on their composition, the materials exhibited a broad range of swelling and adhesion properties, which could be fine-tuned by altering the relative proportion of each constitutional repeat unit.

*In vitro* drug release studies involving pilocarpine or chloramphenicol demonstrated that the materials under consideration may be most appropriate for the delivery of sparingly water-soluble actives.

*In vivo* experiments revealed that the degree of swelling and hydrophobicity of the matrix are key factors in effecting controlled drug release. The inserts induced a more pronounced miotic effect than the eyedrop control, and the period of significant activity increased by up to 110\%. 

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2.5. References


Chapter 3 - Hybrid polymeric networks based on acrylic acid-functionalised chitosan and NIPAM or HEMA, as inserts for ocular drug delivery

3.1. Introduction

As a biocompatible and biodegradable material that also shows good release characteristics, chitosan possesses many of the desired properties for safe use as a pharmaceutical excipient [1-4]. Consequently, much research has concentrated on the use of chitosan as a vehicle for the controlled and targeted release of therapeutic agents [5-9], with many recent publications highlighting the need for chemical functionalisation as the key to optimal carrier properties [10-15]. The hydroxyl moieties and the primary amino group of the D-glucosamine unit represent two readily accessible sites for the attachment of pendent functionalities [16,17]. Such structural modifications both impact upon the physicochemical and biological properties of the material, and allow the manipulation of its morphology [13].

The covalent crosslinking of chitosan affords three-dimensional networks that exhibit desirable drug-diffusion characteristics. Examples of agents that have been used to effect crosslinking include: aldehydes [18-22], itaconic acid [23], naturally-occurring genipin [24], diisocyanates [25], diepoxides [26] and gelatin [27]; thermal crosslinking has also been demonstrated [28]. An alternative crosslinking strategy involves the formation of direct covalent bonds between chitosan and other biocompatible polymers (Fig. 3.1). This approach offers the opportunity for further refinements to the properties of the drug carrier (e.g. mechanical strength; pH and temperature sensitivity), by combining the advantages of the chitosan molecule with those of the partner polymer. While semi- or full- interpenetrating polymer networks (IPN) combining chitosan with synthetic macromolecules have received
considerable attention as controlled release systems [29-33], little is known about the drug-carrier capabilities of analogous hybrid polymer networks (HPN), with reports on chitosan-based systems limited to materials incorporating gelatin [34-36], collagen [37], glyoxal [38] or silylating agents [39]. With the exception of an enzymatically catalysed crosslinking reaction involving chitosan and gelatin [40], crosslinking is normally effected using dialdehydes, which are toxic [41-43].

![Diagram of polymer networks](image)

Fig. 3.1. Covalently crosslinked three-dimensional networks: a) hybrid polymer networks (HPN; chitosan-polymer crosslinks); b) semi-interpenetrating polymer networks (semi-IPN; inter-chitosan crosslinks); c) full-interpenetrating polymer networks (full-IPN; inter-chitosan and inter-polymer crosslinks); and, d) interpolymeric complexes (no crosslinks).

Here we report the results of our study on the synthesis and characterisation of hybrid polymer networks based on acrylic acid-functionalised chitosan (CSf) that is coupled with 2-hydroxyethyl methacrylate (HEMA) [44-48] or with N-isopropylacrylamide (NIPAM) [30,49], and evaluate the ability of these systems to act as vehicles for ophthalmic drug delivery. The performance of the materials is compared with that of interpolymeric complex analogues (Fig. 3.1) that have been obtained by radical-induced polymerisation of HEMA or
NIPAM in the presence of chitosan that had not been subjected to functionalisation. The influences of network structure and composition upon the swelling properties, potential bioadhesion behaviour and drug release characteristics have also been measured, and in vivo experiments (rabbit model) have been performed to establish the release profile of pilocarpine hydrochloride that has been incorporated into these systems.

3.2. Materials and methods

Low molecular weight chitosan ($M_w = 75,000$; deacetylation degree 87.5 %) was used as obtained from Fluka. N-isopropyl acrylamide (Acros) was recrystallised from a mixture of $n$-hexane and toluene (40/60, v/v), while 2-hydroxyethyl methacrylate (Sigma-Aldrich) was purified by passing it through an inhibitor removal column (hydroquinone, Sigma-Aldrich). Pharmaceutical grade therapeutic agents (pilocarpine hydrochloride, Acros; atropine sulphate, Fluka; chloramphenicol and norfloxacin, Sigma-Aldrich) were used as received. Preparation of phosphate buffer solutions (PBS; pH = 7.4; ionic strength 0.497 M) were prepared from sodium monohydrogen phosphate, sodium dihydrogenphosphate and NaCl [50]. All other solvents and chemicals were obtained from Sigma-Aldrich and used as received.

3.2.1. Synthesis of acrylic acid-functionalised chitosan (CSf)

A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC; 5.75 g; 0.03 mol) and acrylic acid (AA; 5 ml; 0.073 mol) in deionised water (50 ml) was allowed to react (room temperature, 1 h) before being mixed (with stirring) with a solution of low molecular weight chitosan (CS) in 0.05 M aqueous hydrochloric acid (1 % w/v; 500 ml). The resulting solution was adjusted to pH 5 (aq. NaOH, 0.5 M) and stirred for 24 h (10 °C) to
yield the crude product, which was purified by continuous dialysis (membrane cut-off 12000 Da) against 0.5 mM hydrochloric acid (5 days) and then against water (3 days). The purity of the freeze-dried product was assessed by $^1$H NMR spectroscopy, and its degree of functionalisation ($DF$) was determined by elemental analysis [51]:

$$DF = \frac{\Delta_{CN}}{3M_{CN}(1-DA)} \times 100$$

where: $\Delta_{CN}$ is the difference between the ratio of found C and N percentages in grafted CSf and the corresponding ratio in the CS sample; $M_{CN}$ is the ratio of C/N molar masses; and $DA$ is the degree of deacetylation of the initial chitosan.

3.2.2. General method for the preparation of hybrid polymer networks

To a stirred solution of AA-functionalised chitosan (CSf; $DF = 67.2 \%$) and synthetic monomer (either HEMA or NIPAM; Table 3.1) in dilute aqueous acetic acid (1 % w/v; 30 ml) was added a mixture (1:3 w/w) of ammonium persulphate and sodium bisulphite (1 % w/w of the acrylic functionality). After stirring (24 h, 25 °C), the hydrogel products were separated by centrifugation, washed (methanol 2x, deionised water 3x) and freeze-dried. For comparison, interpolymeric complexes [ic(CS/PHEMA) and ic(CS/PNIPAM)] with similar composition were prepared following the same procedure but with the AA-functionalised chitosan being replaced by the unmodified polymer (Table 3.1). Minitablets (8 mm in diameter) were prepared by compression (2 tonne, 1 min).

3.2.3. Swelling and adhesion measurements

The swelling properties of the synthesised materials were evaluated at physiological pH (7.4) in PBS, by determining the volume of buffer absorbed by each sample (wafers, Ø 8 mm; 30 mg) in a micro column (QIAquick® Spin Column 50, Ø 10 mm) that was attached
to a micro syringe. Phosphate buffer (1 ml) was injected with a microsyringe into the micro column. The buffer was withdrawn and measured at regular time intervals. The equilibrium degree of swelling, $DS_{eq}$ (% weight), was calculated as follows:

$$DS_{eq} = \frac{v_{eq} - v_0}{v_0} \times 100$$

$v_0$ and $v_{eq}$ are, respectively, the initial and equilibrium sample weights; mean values given for each sample are the result of at least three determinations.

The kinetic parameters of swelling were calculated from the equation used by Peppas [52]:

$$\frac{M_t}{M_\infty} = k_s \times t^n$$

where $M_t$ represents the amount of liquid transferred into the hydrogel at time $t$ and $M_\infty$ is the amount transferred at equilibrium swelling; $k_s$ is the swelling constant, incorporating characteristics of the matrix such as porosity, and $n$ is the diffusion exponent, which is indicative of the mechanism of water uptake.

The adhesion of polymer wafers ($\Omega$ 8 mm; 30 mg polymer; 2 tonne, 1 min) onto hydrated dialysis tubing membrane (cellulose, Visking DTV14000) was evaluated in PBS (pH=7.4) using a Stable Microsystem TATX Plus Analyzer. The parameters used are described already in section 2.2.2. The maximum detachment force (MDF) and the corresponding work of adhesion (TWA) were determined at 20 °C and at 40 °C; pure homopolymers (PHEMA, PNIPAM and low molecular weight chitosan) were used as controls.

### 3.2.4. Drug release in vitro

Drug-containing wafers ($\Omega$ 8 mm; 90 mg) were pressed (2 tonne, 1 min) following physical mixing (pestle and mortar) of each polymer (70 mg) with the appropriate active (20
mg): pilocarpine hydrochloride, chloramphenicol, atropine sulphate or norfloxacin (22.2 %w/w). *In vitro* release profiles under sink conditions (phosphate buffer, pH 7.4; 37 °C) were obtained spectrophotometrically using a dissolution apparatus (Copley DT-70, Erweka Instruments: 500 ml; U.S.P. II Apparatus - paddle method; 25 rpm) connected to a spectrophotometer (UV-300 Spectronic Unicam; pilocarpine - 215 nm, chloramphenicol - 282 nm, atropine - 219 nm, norfloxacin - 323 nm) *via* an automatic flow-through sampling system. At the beginning of each experiment, wafers were introduced (dropped in) at the bottom of the dissolution baths, then the drug release was followed spectrophotometrically. Calibration was performed using standard solutions of drug (0.001 - 0.02 % w/w) in phosphate buffer. Blank experiments, using drug-free minitablets, confirmed that there was no interfering background absorption.

3.2.5. *Drug release in vivo*

Ocular wafers (11.25 mg; Ø 8 mm, 0.25 mm thickness) were prepared by compressing (at 2 tonne, for 1 min) a mixture (obtained using pestle and mortar) of the powdered drug pilocarpine (1.25 mg) and polymer (10 mg) (the mixture contains 11.1 % w/w drug). The induced miotic effect of the ocular wafers was assessed in male, albino New Zealand rabbits (4.0 - 4.5 kg) until disappearance, by timed measurements of the pupillary diameter. As a positive control (using the other eye), corresponding amounts of drug in aqueous solution (*i.e.* 50 µl, containing 1.25 mg pilocarpine) were administered dropwise (2 drops); drug-free minitablets (without loaded drug) provided the blank controls.

Procedures were approved by the Ethical Review Committee at the University of Portsmouth, and all studies were performed in accordance with Home Office regulations for the care and use of laboratory animals.
3.3. Results and discussion

3.3.1. Synthesis and characterisation of hydrogels

Acrylic acid moieties were immobilised onto chitosan chains through amidic bonds, the formation of which was facilitated by water-soluble carbodiimides [53]. Various degrees of functionalisation were obtained, as found by elemental analysis, but only chitosan with a degree of amino group functionalisation of 67.2% was used for further experiments. The immobilised acrylic acid moieties provided a polymerisable anchor that allowed the modified chitosan to participate in radical polymerisation reactions with water-soluble co-monomers without the need to employ crosslinkers (Fig. 3.2).

![Chemical structure of synthetic co-monomers](image)

**Fig. 3.2.** Acrylic acid-functionalised chitosan and synthetic co-monomers used.

Hybrid polymer networks were prepared by the free radical copolymerisation of poly(N-isopropylacrylamide) or poly(2-hydroxyethyl methacrylate) and AA-functionalised...
low molecular weight chitosan (CSf), as described in section 3.2.2. The final composition of the synthesised networks was determined by elemental analysis and is presented in Table 3.1.

In general, lower yields were obtained as the chitosan / HEMA (NIPAM) feed ratio was increased (Table 3.1). At low chitosan feed ratios, interpolymeric complexes (i.e. ic(CS/PHEMA), ic(CS/PNIPAM)) are seen to entrap more chitosan than corresponding hybrid polymer networks. At high feed ratios, there appears to be a limit to the amount of natural polymer that can be incorporated into the intermolecular complex (50–60 %, in ic(CS/PHEMA), ic(CS/PNIPAM)). In three-dimensional hybrid networks, the presence of AA anchors facilitates the incorporation of up to 73 %w/w chitosan into the structure.

Table 3.1. Feed ratio (mass of CSf per 100 g of either HEMA or NIPAM) and final composition (% w/w) of the synthesised hybrid polymer networks, as determined by elemental analysis.

<table>
<thead>
<tr>
<th>Feed ratio (g CSf, or CS, per 100 g synthetic monomer - either HEMA or NIPAM)</th>
<th>Final composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hpn(CSf/HEMA)</td>
</tr>
<tr>
<td></td>
<td>% CSf Yield %</td>
</tr>
<tr>
<td>1 5</td>
<td>13.8 89</td>
</tr>
<tr>
<td>2 10</td>
<td>na 84</td>
</tr>
<tr>
<td>3 20</td>
<td>20.5 74</td>
</tr>
<tr>
<td>4 40</td>
<td>46.6 74</td>
</tr>
<tr>
<td>5 60</td>
<td>na 64</td>
</tr>
<tr>
<td>6 80</td>
<td>64.4 58</td>
</tr>
<tr>
<td>7 100</td>
<td>72.7 47</td>
</tr>
<tr>
<td>8 140</td>
<td>na 34</td>
</tr>
</tbody>
</table>

CS – low molecular weight chitosan; CSf – low molecular weight chitosan modified with acrylic acid; hpn(CSf/HEMA) – hybrid network of AA-modified chitosan with HEMA; hpn(CSf/NIPAM) – hybrid network of AA-modified chitosan with NIPAM; ic(CS/PHEMA) – interpolymeric complex of low molecular weight chitosan and HEMA; ic(CS/PNIPAM) – interpolymeric complex of low molecular weight chitosan and NIPAM; % CSf indicates the low molecular weight chitosan content of the final product; na – data not available.
The proportion of incorporated low-molecular-weight chitosan was also influenced by the chemical structure of the synthetic co-monomer: yields for ic(CS/PHEMA) complexes were higher than for ic(CS/PNIPAM) structures. The formation of interpolymeric complexes (ic(CS/PHEMA), ic(CS/PNIPAM)) appeared to be favoured by the hydrogen-bonded interactions between the OH groups of HEMA and the hydrophilic chitosan chains; in the case of NIPAM, such inter-chain interactions are probably suppressed by the hydrophobicity of the isopropyl moieties. By contrast, hpn(CSf/NIPAM) hybrid polymer networks were obtained in better yields than corresponding hpn(CSf/HEMA) structures, most probably due to favourable interactions between immobilised AA and NIPAM. While, at low concentrations of AA-functionalised low molecular weight chitosan (CSf), the modified biopolymer acts as a crosslinker for PNIPAM or PHEMA, at high concentrations it provides the skeleton of a network that is crosslinked by short chains of synthetic polymer (PNIPAM or HEMA); increasing the CSf to NIPAM (HEMA) ratio suppresses crosslinking, as is demonstrated by the observed trends in corresponding yields, Table 3.1.

3.3.2. Swelling properties of the synthesised materials

Under simulated physiological conditions (37.5 °C, pH 7.4), the synthesised materials exhibited increased equilibrium degrees of swelling with increasing amounts of incorporated chitosan. Hybrid networks swelled to a greater extent and, also, reached maximum swelling more rapidly than the corresponding interpolymeric complexes, with the NIPAM-containing hybrid network hpn(CSf/NIPAM)_8 exhibiting the most pronounced increase (1150 %, Fig. 3.3). At 37.5 °C both types of NIPAM-containing materials showed very limited swelling at low chitosan content, indicating the collapse of the PNIPAM chains and that hydrophobic interactions become dominant – PNIPAM in aqueous solution is known to exhibit a temperature-controlled (~31 °C) conformational transition from random coil to hypercoil.
In some cases, the wafers with high chitosan content (CS > 100, Table 3.1) – especially those of interpolymeric complexes containing NIPAM – disintegrated in the phosphate buffer solution (PBS).

![Bar chart showing degree of swelling as a function of chitosan content](image)

**Fig. 3.3.** The degree of swelling at equilibrium as a function of chitosan content (CSf = low molecular weight chitosan modified with acrylic acid, HEMA = hydroxy ethyl methacrylate, NIPAM = N- isopropylacrylamide).

In all cases, the rate of swelling increased with increasing chitosan content. This effect was more pronounced with NIPAM-containing hybrid networks than with other materials (Table 3.2).

For materials with low proportions of chitosan, the diffusion kinetic exponent of time $n$ (section 3.2.3) is seen to adopt values that are close to 0.5, suggesting a Fickian transport process in which water diffusion into the polymeric network becomes markedly more significant than network relaxation, and represents the dominant mechanism controlling swelling.
Table 3.2. Kinetic parameters of swelling for selected interpolymeric complexes and hybrid polymeric networks (\(k_s\) – swelling rate constant; \(n\) – diffusion exponent; \(n_{\text{exp}} > 3\) measurements; values shown represent the mean; ± values represents standard deviation).

<table>
<thead>
<tr>
<th>Feed ratio CSf or CS - g per 100 g of monomer (either HEMA or NIPAM)</th>
<th>hpn(CSf/HEMA)</th>
<th>hpn(CSf/NIPAM)</th>
<th>ic(CS/PHEMA)</th>
<th>ic(CS/PNIPAM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(k_s [h^{-n}])</td>
<td>(n)</td>
<td>(k_s [h^{-n}])</td>
<td>(N)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0.312 ± 0.005</td>
<td>0.435 ± 0.003</td>
<td>0.232 ± 0.017</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.421 ± 0.015</td>
<td>0.524 ± 0.010</td>
<td>0.455 ± 0.023</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>0.456 ± 0.022</td>
<td>0.555 ± 0.015</td>
<td>0.635 ± 0.045</td>
</tr>
<tr>
<td>8</td>
<td>140</td>
<td>0.564 ± 0.047</td>
<td>0.665 ± 0.007</td>
<td>0.834 ± 0.010</td>
</tr>
</tbody>
</table>

Increased proportions of chitosan in the network are reflected by anomalous transport mechanisms (0.5 \(n\) < 1.0); the swelling behaviour of NIPAM-containing hybrid polymer networks hpn(CSf/NIPAM) appears to be more sensitive to the amount of incorporated chitosan than that of HEMA-containing materials.

### 3.3.3. Adhesion characteristics

Several methods are available for the evaluation of the adhesive strength of pharmaceutical formulations [55, 56]. In preliminary experiments designed to evaluate the adhesive potential of our materials, hydrated cellulose dialysis membrane was employed as the model test surface [57, 58]. Although large intersample variations were evident, tensile strength measurements (method: section 2.2.2) indicated that, at room temperature (20 °C), the NIPAM-containing polymers were up to 10\(\times\) more adhesive than HEMA-based materials.
Fig. 3.4. Adhesion data for hybrid polymeric networks (HPN), interpenetrating polymeric networks (IPN) and pure polymers: a) maximum detachment force [MDF]; b) total work of adhesion [TWA]; bars represent standard errors. (n_{exp}=6; temperature 20 °C)
There appears to be an inverse relationship between the chitosan content of the formulation and its adhesive strength (both the detachment force and the work of adhesion were suppressed by increasing proportions of chitosan in the formulation (Fig. 3.4). Of all materials tested, the adhesiveness of the HEMA-containing interpolymeric complex ic(CS/PHEMA) was least sensitive to the amount of chitosan present. This is consistent with the proposed weak interactions between low-molecular-weight chitosan and the cellulose membrane. No significant differences in the adhesive behaviour could be identified between the interpolymeric complexes and the hybrid polymer networks of analogous composition.

Fig. 3.5. Effect of temperature on adhesive properties of selected materials (MDF; TWA; bars are standard errors): measurement at 20 °C and 40 °C (n_{exp}=6).

In view of the fact that the physiologically relevant temperature of the ocular surface is highly sensitive to environmental conditions, a study of adhesive potential was also
conducted at 40 °C: NIPAM-containing materials exhibited decreased adhesion at this temperature (Fig. 3.5).

3.3.4. In vitro release

For the in vitro release studies, cholinergic agonists and antagonists (pilocarpine and atropine) and two broad-spectrum antibacterial agents (chloramphenicol and norfloxacin) were selected for investigation (Fig. 3.6). Since prolonged retention with suppressed discomfort and minimal disruption of vision are critical requirements for ocular inserts, materials for further studies were selected from polymer networks that showed adhesive properties that were coupled with limited swelling. For these materials, the release of each drug was monitored over time; at least five experiments were performed in each case, with results presented as averages.

![Drug structures](image)

Fig. 3.6. Drug structures: a) pilocarpine hydrochloride; b) chloramphenicol; c) atropine sulphate; and, d) norfloxacin.
Fig. 3.7 (a, b). Chloramphenicol release profiles from: a) HEMA-containing hybrid polymeric networks; and, b) HEMA-containing interpolymeric complexes; (20 mg chloramphenicol in 70 mg of polymer; PBS, pH=7.4; 37 °C; n_{exp} ≥ 5; means represented, standard deviations were < 12 %).
Fig. 3.7 (c, d). Chloramphenicol release profiles from: c) NIPAM-containing hybrid polymeric networks; and, d) NIPAM-containing interpolymeric complexes; (20 mg chloramphenicol in 70 mg of polymer, PBS, pH=7.4; 37 °C; n_{exp} ≥ 5; means represented, standard deviations were < 12 %).
In general, materials based on HEMA and AA-modified chitosan released their chloramphenicol (CHF) content at a slower rate than those based on NIPAM, with some materials retaining a small amount of the active even after 24 h (Fig. 3.7). hpn(CSf/NIPAM)_1, which showed the slowest release of chloramphenicol, was the exception.

The release rates are seen to correlate well with the equilibrium degree of swelling ($DS_{eq}$): materials with low $DS_{eq}$ (200-400%) and low $k$ values (0.30-0.56 h$^{-n}$) – i.e. hpn(CSf/HEMA)$_1$, hpn(CSf/HEMA)$_3$, hpn(CSf/NIPAM)$_1$, ic(CS/PHEMA)$_3$ – appear to be most suitable for applications demanding the slow release of chloramphenicol.

Many of the interpolymeric complex wafers that were based on NIPAM [ic(CS/PNIPAM)] released their chloramphenicol content in less than 3 h but, interestingly, only about 50% of the loaded drug was seen to be released from NIPAM-based materials that contained very low proportions of chitosan [hpn(CSf/NIPAM)$_1$, ic(CS/PNIPAM)$_1$, ic(CS/PNIPAM)$_2$]; probably due to strong hydrogen bonding between this drug and PNIPAM.

The capability of the materials to effect the controlled release of atropine sulphate, norfloxacin and pilocarpine hydrochloride was also considered; the data are presented in Figs. 3.8 and 3.9. The release of atropine sulphate – a water-soluble drug – was rapid; only HEMA-based interpolymeric matrices appeared to exert some influence over the release kinetics of this active. By contrast, norfloxacin exhibited prolonged release profiles (extending to over 48 h) with almost all materials considered.

The compromise between the strength of the carrier-drug interactions and the solubility of the active is an important issue in drug release, as is exemplified by the behaviour of the water-soluble ionic compounds pilocarpine hydrochloride and atropine sulphate, both of which are associated with rapid release rates (Fig. 3.9).
Drug release from hydrophilic matrices is influenced by protean contributions from the processes of swelling, diffusion and erosion [59-61], and its assessment is complicated by the limitations of the in vitro evaluation procedure, as is highlighted by the release profiles presented in Figs. 3.8 and 3.9, some of which appear to indicate that more drug is released than is actually present in the formulation.
Fig. 3.9. Comparison of in vitro release profiles from three types of HEMA-based wafers: CHF - chloramphenicol; ATP - atropine sulphate; NFX - norfloxacin; P - pilocarpine hydrochloride. (20 mg chloramphenicol in 70 mg of polymer; PBS, pH=7.4; 37 °C; n_{exp} ≥ 5; means represented, standard deviations were < 12 %).
Drugs with low water solubility (chloramphenicol, norfloxacin; aqueous solubility < 2.5 mg/ml at 25 °C) exhibited prolonged release profiles when formulated into HEMA-based vehicles [e.g. hpn(CSF/HEMA)_3, ic(CS/PHEMA)_4].

Drug-polymer interactions, network porosity and degree of hydrophilicity of the matrix are also important factors influencing controlled release. Nonetheless, in accord with previous findings [62], high release rates were obtained with pilocarpine (Fig. 3.9) – a water-soluble compound that does not possess protons capable of participating in hydrogen bonding (Fig. 3.6).

3.3.5. In vivo experiments

The rabbit is widely regarded as the ocular model of choice prior to human trials [63]. The readily accessible New Zealand albino rabbit model was employed for our in vivo studies (two animals; 48 h rest periods between experiments).

As a positive control, an aqueous solution of pilocarpine hydrochloride was also administered (50 µl, containing 1.25 mg drug); drug-free minitablets (blank controls) confirmed that the pure hydrogels did not contribute to the observed miotic effect. The variation in pupillary diameter was monitored over time using a standard pupillary diameter gauge. For each material, the presented values are averages from at least six experiments. Statistical treatment of the results (ANOVA, two-factors with replication; 6 replicates; p < 0.05) indicated a significant difference between some of materials [hpn(CSF/HEMA)_1, hpn(CSF/HEMA)_3, ic(CS/PHEMA)_3] and control solution, and a significant interaction between some of materials [hpn(CSF/HEMA)_1, hpn(CSF/HEMA)_3, ic(CS/PHEMA)_3] and time.

All inserts tested induced a relatively rapid (< 30 min) decrease in pupillary diameter, to a minimum value. This was followed by a much slower “pupillary diameter increase”
stage. The end of each experiment was signalled by the pupil returning to its original size (Fig. 3.10).

**Fig. 3.10.** *In vivo*, pilocarpine-induced miotic effect (control: aqueous pilocarpine solution - 1.25 mg pilocarpine) in rabbit; n exp ≥ 6, bars represent standard errors.
In most cases, the inserts induced a more pronounced miotic effect than the eyedrop control, with the period of significant activity \((t_{1/2})\) increasing by up to 57\% (Table 3.3). The effect of pilocarpine, as gauged by the AUC values, was more pronounced when this active was released from hybrid polymer networks than from interpolymeric complexes. Overall, HEMA-based materials performed better than their NIPAM-containing analogues, with hpn(CS/HMA)_3 representing the most promising carrier vehicle.

Table 3.3. *In vivo* evaluation of the pilocarpine-induced miotic effect in rabbits (data from Fig. 3.10).

<table>
<thead>
<tr>
<th>Materials</th>
<th>MP [mm]</th>
<th>(t_{\text{max}}) [min]</th>
<th>(t_{1/2}) [min]</th>
<th>(t_{\text{e}}) [min]</th>
<th>AUC [mm × min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>hpn(CS/HMA)_1</td>
<td>2.1</td>
<td>15</td>
<td>220</td>
<td>390</td>
<td>413</td>
</tr>
<tr>
<td>Control</td>
<td>1.7</td>
<td>30</td>
<td>140</td>
<td>300</td>
<td>244</td>
</tr>
<tr>
<td>hpn(CS/HMA)_3</td>
<td>2.3</td>
<td>10</td>
<td>200</td>
<td>390</td>
<td>463</td>
</tr>
<tr>
<td>Control</td>
<td>1.8</td>
<td>30</td>
<td>135</td>
<td>300</td>
<td>244</td>
</tr>
<tr>
<td>hpn(CS/NIPAM)_1</td>
<td>1.8</td>
<td>15</td>
<td>110</td>
<td>360</td>
<td>278</td>
</tr>
<tr>
<td>Control</td>
<td>1.7</td>
<td>30</td>
<td>140</td>
<td>270</td>
<td>231</td>
</tr>
<tr>
<td>ic(CS/PHEMA)_3</td>
<td>1.9</td>
<td>30</td>
<td>170</td>
<td>390</td>
<td>346</td>
</tr>
<tr>
<td>Control</td>
<td>1.8</td>
<td>30</td>
<td>125</td>
<td>330</td>
<td>230</td>
</tr>
<tr>
<td>ic(CS/PHEMA)_4</td>
<td>2.1</td>
<td>10</td>
<td>140</td>
<td>360</td>
<td>303</td>
</tr>
<tr>
<td>Control</td>
<td>2.0</td>
<td>30</td>
<td>110</td>
<td>330</td>
<td>238</td>
</tr>
</tbody>
</table>

*MP* - maximum pupillary response; *\(t_{\text{max}}\)* - time to reach MP; *\(t_{1/2}\)* - MP half time; *\(t_{\text{e}}\)* - total duration of miotic effect; *AUC* - area under the miosis-time profile. hpn - hybrid polymeric network; ic - interpolymeric complex.
3.4. Conclusions

A number of hybrid polymer networks were prepared by the free-radical induced copolymerisation of acrylic acid-functionalised low-molecular-weight chitosan with either 2-hydroxyethyl methacrylate or N-isopropylacrylamide, and their potential to act as mucoadhesive drug-release devices was compared with that of interpolymeric complexes of similar composition. The materials exhibited a broad range of swelling and adhesion properties, which could be refined by altering the relative proportion of the compositional units. In vitro drug release studies involving broad-spectrum antibacterials (chloramphenicol, norfloxacin), cholinergics (pilocarpine) and anticholinergics (atropine) demonstrated that the materials under consideration are promising candidate vehicles for the delivery of sparingly water-soluble actives, particularly norfloxacin. In vivo experiments were complementary in that they confirmed that the degree of swelling and hydrophilicity of the matrix are key factors governing the ability of these materials to act as controlled release vehicles for the ocular delivery of therapeutic agents. HEMA-containing hybrid polymer networks appear to be especially promising.

3.5. References


4.1. Introduction

Underpinned by the principle that well-defined polymeric structures often degrade/disintegrate in a controlled manner to release entrapped therapeutic agents, colloidal drug delivery systems (microparticles, liposomes or nanoparticles) represent an attractive means for improved drug delivery. Such systems may be appropriate hosts for poorly soluble drugs or as carriers for long acting injectable depot formulations and for specific drug targeting. Their drug-release kinetics are influenced by several factors, including: size, nature of the polymeric material, hydrophilicity of the drug, drug loading capacity, method of preparation, and rate of biodegradation.

Amongst the nano-sized particles, nanospheres (matrix systems in which the drug is physically and uniformly dispersed) and nanocapsules (vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane) represent two drug delivery systems of considerable promise [1]. Such systems can be prepared from preformed polymers or directly from monomers through the use of appropriate polymerisation methodology. Techniques involving the polymerization of monomers may utilise the dispersed phase of an emulsion, an inverse microemulsion or a liquid medium that dissolves the monomer but acts as a non-solvent for the polymer. The properties and release characteristics of nanospheres and nanocapsules are often sensitive to their adopted method of preparation.
Table 4.1. Manufacturing techniques used for preparing nanoparticles.

<table>
<thead>
<tr>
<th>Manufacturing process</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation or crosslinking of macromolecules in emulsion form</td>
<td>Burgess et al. [2]</td>
</tr>
<tr>
<td>Interfacial polymerisation</td>
<td>Duc-Mauger et al.  [3]</td>
</tr>
<tr>
<td>Formation in an aerosol phase</td>
<td>Przyborowski et al. [4]</td>
</tr>
<tr>
<td>Desolvation</td>
<td>Zimmer et al. [5]</td>
</tr>
<tr>
<td>Aggregation by pH adjustment and heat treatment</td>
<td>Taplin et al. [6]</td>
</tr>
<tr>
<td>Formation of nanoparticles via microemulsions</td>
<td>Dechow et al. [7]</td>
</tr>
</tbody>
</table>

Starting from preformed polymers, nanoparticles may be prepared by the precipitation of synthetic polymers, or by denaturation or gelation of natural macromolecules (Table 4.1). Common approaches employ the emulsification of a water-immiscible organic solution of the polymer in a surfactant-containing aqueous phase, followed by solvent evaporation, or by precipitation after the addition of a non-solvent [8].

The next sections present a short review of the nanoparticulate systems considered so far for drug delivery applications, with examples of polymers and surfactants used in ophthalmic nano-formulations; a special attention is paid to biodegradable materials.

Synthesis and characterisation of nanoparticles consisting of poly(vinylpyrrolidinone-co-methacrylic acid) linear chains that are crosslinked via biodegradable short polysaccharidic units, and their in vitro and in vivo pilocarpine release behaviour, are described next. Also, preliminary studies of the potential transport of the nanoparticles through rabbit cornea using $^{14}$C-radiolabelled congeners are presented.
4.1.1 Nanoparticles as drug delivery vehicles

Several studies have shown that, even under conditions of rapid corneal clearance, nanoparticles can facilitate increased drug bioavailability and decreased systemic side effects [9]. The advantages of nanoparticulate drug delivery originate from two of their features. Firstly, because of their small size, nanoparticles penetrate small capillaries and are taken up by cells, allowing efficient drug accumulation at the target sites. Secondly, the use of biodegradable materials for nanoparticle preparation allows sustained drug release within the target site over a period of days or even weeks [9]. Thus, nanoparticles provide an effective drug delivery mechanism for drugs whose targets are cytoplasmic [10].

The development of nanoparticulate delivery systems for targeted drug delivery has been reviewed by Moghimi et al. [11]. Targeted delivery can be achieved by either active or passive targeting: active targeting of a therapeutic agent is achieved by conjugating the therapeutic agent or the carrier system to a tissue or cell-specific ligand. Passive targeting involves the coupling of the therapeutic agent to a macromolecule that passively reaches the target organ. Therapeutic agents encapsulated in nanoparticles or drugs coupled to macromolecules address the target tissue through an enhanced permeation and retention effect. An alternative approach involves the infusion of nanoparticle suspension to an accessible target organ or tissue using catheters. Also, localized delivery of nanoparticles in restenosis may prove a useful strategy.

Nanoparticles are also useful for the delivery of pharmaceutical agents by binding to target cellular epitopes through a ‘contact facilitated drug delivery’ mechanism. Binding and close apposition to the targeted cell membrane permits enhanced lipid–lipid exchange with the lipid monolayer of the nanoparticle, which accelerates the convective flux of lipophilic drugs (e.g. paclitaxel), dissolved in the outer lipid membrane of the nanoparticles, into the
targeted cells. Such nanosystems can serve as drug reservoirs, exhibiting prolonged release kinetics and long persistence at the site.

Another characteristic function of nanoparticles is their capability to transport drugs across several biological barriers. Delivery to the brain of a wide variety of drugs, such as antineoplastics and anti-HIV drugs is markedly hindered by the blood–brain barrier (the primary barrier to the passage of compounds from the blood to the brain, represented mainly by the brain capillary endothelial cells). The application of nanoparticles to brain delivery is a promising way of overcoming this barrier: it has been demonstrated that poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 are effective in transporting the hexapeptide dalargin and other agents into the brain [12].

A combination of mucoadhesive polymers and nanoparticles may provide a means for prolonging residence time at the precorneal area. Endocytosis through the corneal epithelium depends on the particle size and size distribution of the particles: nanoparticles have been found in corneal cells, but the presence of microspheres has not been reported [13].

Thermo- and pH-responsive, and biodegradable nanoparticles comprised of poly(D,L-lactide)-graft-poly(N-isopropylacrylamide-co-methacrylic acid) have been developed by grafting biodegradable poly(D,L-lactide) onto N-isopropylacrylamide and methacrylic acid. A core-shell type nano-structure was formed with a hydrophilic outer shell and a hydrophobic inner core, which exhibited a physiologically relevant phase transition temperature above 37 °C. The drug loading level of 5-fluorouracil (5-FU) encapsulated in the poly(D,L-lactide)-graft-poly(N-isopropylacrylamide-co-methacrylic acid) nanoparticles was as high as 20% and its release was strongly controlled by the pH of the medium [14]. Graziacascone et al. [15] published a comparative study on the encapsulation of lipophilic drugs in poly(D,L-lactic/glycolic acid) nanoparticles and the entrapment the particles in polyvinyl alcohol hydrogels. Release rates from hydrogels loaded with the particles were comparable with
delivery rates from \text{poly}(D,L\text{-lactic/glycolic acid}) \text{ particles, implying that particle size controls the release rates.}

Ophthalmic use of a dispersed system of nanoparticles may help overcome the problems of rapid elimination after instillation and low bioavailability associated with topical eye drops, due to the defence mechanisms of the eye \text{(i.e. reflex blinking, lachrymation and an increased tear flow), and the barrier function and low pain threshold of the cornea} [16,17]. To be effective, the particles must be retained in the ocular cul-de-sac, and the entrapped drug must be released from the particles at an appropriate rate. If the drug leaks out of the particles too fast, then there will be little sustained drug release. If release is too slow, the concentration of the drug in the tears may be too low to allow adequate drug penetration into ocular tissues. To enhance particle retention in the ocular cul-de-sac it is desirable to fabricate the particles from bioadhesive materials. In all cases, one key requirement is that the formulation must be capable of being sterilised.

\textbf{4.1.2 Polymers and surfactants used in nanoparticulate ophthalmic formulations}

The use of various biodegradable polymeric particles has been investigated (Table 4.2). Among these, \text{poly}(D,L\text{-lactic/glycolic acid}) and \text{poly}(D,L\text{-lactic acid}), whose degradation rate depends on average molecular weight and conformation, are pharmaceutically accepted materials [18]. Consequently, \text{poly}(D,L\text{-lactic/glycolic acid}) / \text{poly}(D,L\text{-lactic acid})\text{-nanoparticles offer considerable promise as vehicles for specific targeting and sustained delivery} [19,20].

When surfactant is used, its preferential adsorption at the surface of the particle allows the modification of the physico-chemical properties at the interface. While the surfactant concentration of emulsions is typically of the order of 0.1 \%_{w/w}, in microemulsions it accounts for at least 10 \%_{w/w} of the total content due to the much greater
interfacial area between the aqueous and oily phases. However, such high concentrations of surfactants can impact on ocular toxicity. Since ionic surfactants are generally too toxic for use in such applications, non-ionic surface active agents (poloxamers, polysorbates, polyethylene glycol, tyloxapol) are generally preferred [40, 41].

Table 4.2. Nano- and microparticulate carriers for ophthalmic use.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(butyl)cyanoacrylate</td>
<td>amikacin</td>
<td>Alonso et al. [21]</td>
</tr>
<tr>
<td>Poly(epsilon)caprolactone</td>
<td>betaxolol</td>
<td>Marchal-Heussler et al.</td>
</tr>
<tr>
<td>Poly(isobutyl)cyanoacrylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(epsilon)caprolactone</td>
<td>cartiolol</td>
<td>Maincent et al. [23]</td>
</tr>
<tr>
<td>Poly(lactic acid)</td>
<td>chloramphenicol</td>
<td>Shell et al. [24]</td>
</tr>
<tr>
<td>Albumin</td>
<td>hydrocortisone</td>
<td>Zimmer et al. [25]</td>
</tr>
<tr>
<td>Poly(epsilon)caprolactone</td>
<td>indomethacin</td>
<td>Mason et al. [26]</td>
</tr>
<tr>
<td>Albumin</td>
<td>pilocarpine</td>
<td>Zimmer et al. [5]</td>
</tr>
<tr>
<td>Cellulose acetate hydrogen phthalate</td>
<td></td>
<td>Boye et al. [27]</td>
</tr>
<tr>
<td>Gelatine</td>
<td></td>
<td>Gurny et al. [28]</td>
</tr>
<tr>
<td>Poly(butyl)cyanoacrylate</td>
<td></td>
<td>Leucuta et al. [29]</td>
</tr>
<tr>
<td>Poly(hexyl)cyanoacrylate</td>
<td></td>
<td>Harmia et al. [30,34]</td>
</tr>
<tr>
<td>Polylactic acid</td>
<td></td>
<td>Kreuter et al. [31]</td>
</tr>
<tr>
<td>Poly(methyl)methacrylate-acyclic acid copolymer</td>
<td></td>
<td>Vidmar et al. [32]</td>
</tr>
<tr>
<td>Poly(methyl)methacrylate</td>
<td></td>
<td>Andermann et al. [33]</td>
</tr>
<tr>
<td>Polyamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyphthalamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(hexyl)cyanoacrylate</td>
<td>progesterone</td>
<td>Kreuter et al. [37]</td>
</tr>
<tr>
<td>Poly(butyl)cyanoacrylate</td>
<td></td>
<td>Li et al. [38]</td>
</tr>
<tr>
<td>Poly(alkyl)cyanoacrylate</td>
<td>timolol</td>
<td>Harmia et al. [39]</td>
</tr>
</tbody>
</table>

Sorbitan fatty acid esters (Spans) and polyoxyethylene sorbitan fatty acid esters (Tweens) have found many pharmaceutical applications. They are often used to improve the stability of water-in-oil-in-water (W/O/W) multiple emulsions, which are potentially useful
vehicles for encapsulating water-soluble pharmaceutical compounds or nutrients. Multiple emulsion systems usually require at least two surfactants: a relatively hydrophobic surfactant to stabilize the W/O emulsions and a relatively hydrophilic surfactant to stabilize the O/W emulsions. Combined Spans and Tweens work well for this purpose, even though the individual surfactants do not produce stable multiple emulsion systems.

Surfactants stabilize emulsions by lowering the interfacial tension and by facilitating the formation of electrical or mechanical barriers. When a rigid interfacial complex forms at the primary W-O interface, emulsion stability is enhanced. Improved stability can also be achieved by maximizing the interfacial strength [42].

Due to their low toxicity, amphoteric surfactants, e.g. lecithin, are also of interest. However, the chemical nature of such compounds is highly variable depending on their origins, purity (variation of fatty acids content), composition and concentration of phospholipids. For example, lecithin from egg yolk contains 21% of phosphatidylcholine, 22% of phosphatidylethanolamine and 19% of phosphatidylinositol.

Miranol MHT® (an aqueous solution containing 3.4 %w/w of NaCl and 34.5 %w/w of lauroamphodiacetate), is another amphoteric surfactant that is tolerated well by the eye [43].

4.1.3 Biodegradable polymers for drug delivery to the eye

Polymer biodegradation is a process in which a deterioration in the properties of a material takes place due to factors such as temperature, light, radiation, presence of enzymes etc. Although they may arouse some immune response, biodegradable materials are important for many biomedical applications, including wound healing, tissue reconstruction and controlled drug delivery. Their main advantage is that it is often possible to control their
degradation rate, which, in turn, impacts upon the length of time over which the material can remain in contact with living tissue [44].

Williams, who defined biodegradation as a biological cleavage of the polymeric structure, proposed three types of mechanism that lead to dissolution by erosion [45]:

1. erosion of (water-soluble) polymers that are made initially insoluble via crosslinking with hydrolytically unstable crosslinks (type-1);

2. erosion of linear polymers, which are initially water insoluble and which become solubilized by hydrolysis, ionization or protonation of a pendant group, but without backbone cleavage (type-2); and,

3. erosion of polymers that are water insoluble and hydrolyse to small soluble molecules by backbone cleavage (type-3);

Erosion does not necessarily yield molecules of a sufficiently small size to be eliminated from the body, and currently there is no universal agreement as to the use of the terms "bioerodible" and "biodegradable".

Erosion of polymers does not necessarily proceed by a single mechanism. Generally, systems in an aqueous biological environment undergo erosion through hydrolysis. The presence of enzymes often significantly enhances the rate of erosion [46-48].

Polymers susceptible to type-1 erosion e.g. gelatin, collagen, polyacrylamides, poly(vinylalcohol) and poly(vinylpyrrolidone) are normally water soluble materials that have been rendered insoluble by crosslinking. Such materials are considered unsuitable hosts for water soluble, low molecular weight species [49], but useful for the release of sparingly water-soluble actives (chloramphenicol, hydrocortisone acetate) [50] or water-soluble macromolecules such as enzymes, proteins and antigens [51-53], which can be immobilized in the hydrophilic matrix by physical entanglement.
In type-2 erosion no significant change to the molecular weight of the polymer takes place. Unless the erosion process produces water-soluble materials with molecular weights below that of the renal threshold (the concentration of a substance dissolved in the blood above which the kidneys begin to remove it into the urine), such materials cannot be employed in biomedical applications. Heller and coworkers [49] demonstrated, using rabbits, the usefulness of poly(vinylmethylether/maleic anhydride) disks containing hydrocortisone as an ophthalmic delivery system: zero-order release of hydrocortisone was achieved. Trials with pilocarpine, a highly water soluble substance, were carried out by Urtti and coworkers [54, 55]: by increasing the size of the alkyl ester moiety, the hydrophobicity of the polymeric matrix was controlled and a constant release of pilocarpine was achieved. The erosion of poly(vinylmethylether/maleic anhydride) occurs via the ionisation of carboxylic acid functionalities, which generates hydrogen ions and causes a localised decrease in pH at the surface of the matrix [56].

Type 3 erosion leads to the formation of small, soluble molecules, resulting from the cleavage of the polymeric chains. As small molecules can be eliminated easily from the body, this mechanism conforms with the definition of biodegradability proposed by Graham and Wood [57]. Provided that degradation products are not toxic, polymers undergoing type 3 erosion are particularly well suited as carriers for the systemic administration of active agents. Such biodegradable systems have several advantages over conventional ophthalmic delivery systems in that they can be applied directly to the surface of the eye or can be used as intraocular implants. The early use of poly(cyanoacrylate)s as tissue adhesives in surgical procedures and in the treatment of corneal perforations has now been extended to their use as drug-carrier nanoparticles [58]. A major advantage of poly(alkylcyanoacrylate) nanoparticles is their biodegradability [59], which occurs via surface erosion through enzymatic hydrolysis.
Poly(lactic acid), poly(glycolic acid) and their copolymers are biodegradable, by hydrolysis to release lactic and glycolic acids, which are eliminated from the body via the Krebb’s cycle [47, 60]. The biodegradation of these polymers is influenced by the configuration of the lactic acid used, and by the proportion of lactic and glycolic acid in the copolymer. Moritera and coworkers [61] prepared - by solvent evaporation - poly(D,L-lactic acid) and poly(D,L-lactic/glycolic acid) microspheres (diameter = 50 μm), which were loaded with 5-fluorouracil (an agent used for inhibiting cellular proliferation in vitreoretinopathies). When the microspheres were injected into rabbit eyes, a release of 5-Fluorouracil over 2-7 days was achieved, depending on the polymer used and its molecular weight. Kimura et al. [62] used poly(D,L-lactic acid) microspheres loaded with adriamycin to prevent proliferation of fibroblasts after glaucoma-filtering surgery. After a subconjunctival injection, release of the antimetabolite, extending over a period of 20 days, effected a decrease in intraocular pressure between the 6th and the 16th day after injection, and maintained the filtration bleb for 15 days. Khoobehi and coworkers [63] studied the release of fluorescein sodium salt from poly(D,L-lactic acid) and poly(D,L-lactic/glycolic acid) microspheres and examined the kinetics of the process following subconjunctival and intravitreous injections. Topical applications of poly(D,L-lactic acid) nanoparticles loaded with indomethacin [26] and topical application of poly(D,L-lactic/glycolic acid) nanoparticles loaded with betaxolol have also been described [22]. In both cases, the observed increase in ocular bioavailability was explained in terms of the accumulation of nanoparticles in the conjunctival cul-de-sac.

Unlike certain poly(D,L-lactic acid) and poly(D,L-lactic/glycolic acid) polymers, poly(γ-caprolactone) hydrolyses at very low rates. This slow hydrolysis has been utilised in the development of the contraceptive implant capronor [64]. Hydrolysis produces γ-hydroxycaproic acid by cleavage of the polymeric chains at the ester linkage. This random
cleavage of the polymeric chains leads initially to a decrease of molecular weight, without
significant weight loss, but when $M_n$ reaches about 5000, small polymeric fragments begin
to diffuse from the matrix. These fragment further and undergo phagocytosis [65]. Another
study showed that poly($\gamma$-caprolactone) nanoparticles loaded with betaxolol base are more
effective for intraocular pressure control than nanoparticles loaded with betaxolol
hydrochloride [22]. This enhanced effect has been explained in terms of the efficient
agglomeration of poly($\gamma$-caprolactone) nanoparticles in the conjunctival cul-de-sac and the
use of the active principle in its basic form.

Heterogeneous erosion from the surface accompanied by near zero order degradation
kinetics has been observed with polyanhydrides composed of hydrophobic backbones that
incorporate alternating hydrophilic anhydride linkages. Polyanhydrides seem to be promising
biocompatible materials for medical applications: neither the polymers nor their breakdown
products provoked inflammatory responses to the cornea of rabbits over six weeks. Carriers
of 1,3-bis($p$-carboxyphenoxy) hexane copolymerised with sebacic acid are also reported to
be useful for the controlled delivery of antimetabolites after glaucoma filtering surgery [66].

While polyesters such as those of lactic acid and glycolic acid are hydrophilic and
hydrolytically biodegradable polymers, poly(ortho esters) are hydrophobic polymers, which
under certain conditions can undergo a heterogeneous erosion process confined at the
polymer-water interface. Because all poly(oxyethylene)s contain pH-sensitive linkages in
their backbone, they are stable in alkaline media but susceptible to acid-catalysed hydrolysis.
The erosion rate can be controlled by the incorporation of acidic or basic excipients into the
polymer [67]. Because of promising biocompatibility, the semi-solid poly(oxyethylene) is
considered to be a useful injectable bioerodible polymer for the controlled release of 5-
fluorouracil and mitomycin C in glaucoma filtering surgery [66].
Polysaccharides represent a rich source of biocompatible gels that are widely used, in their natural structure or as semi-synthetic derivatives, in ophthalmic preparations. Most of these materials are water soluble, a property that can be controlled by the degree of crosslinking. Since the first use of methylcellulose in ophthalmic preparations (in the 1940s) its use has span eye drops, artificial tears, contact lens solutions and inserts. Higher concentrations of cellulose generally increase the bioavailability of a drug, but negative effects, such as irritation, are also increased. Nonetheless, hydroxyethyl cellulose is reported to show generally better tolerability than hydroxy propyl methyl cellulose or hydroxy propyl cellulose [66].

Hyaluronic acid has several uses in ophthalmology. Beside topical ocular drug delivery, it has been used as a tear substitute in the treatment of dry eye, for protecting the corneal endothelial cells during intraocular surgery and as a replacement for vitreous humour in humans. Owing to its mucoadhesive properties, hyaluronic acid increases the precorneal residence time and may also have a bioavailability enhancing effect [66].

Chondroitin sulfate is a polysaccharide that is comparable to hyaluronic acid in terms of its viscoelasticity or its capacity to protect corneal endothelium. However, unlike hyaluronic acid, which is a highly viscous non-Newtonian fluid, chondroitin sulfate has low viscosity and displays Newtonian behaviour. Chondroitin sulphate-containing preparations have been investigated for the treatment of dry eyes: isotonic saline solutions containing 1% chondroitin sulfate are well tolerated. A proposed use of Chondroitin sulfate as a viscous agent in anterior segment surgery has proved unsatisfactory as it causes a temporary increase in intraocular pressure and is characterised by a high elimination rate [66].

Due to its mucoadhesive nature, permeability enhancing properties and biodegradability by lysozyme, chitosan represents another potentially useful polymer for the formulation of micro- and nanoparticles, [68, 69]. Chitosan micro- or nanoparticles are
reported to have a higher precorneal retention than chitosan solutions, and depending on size, such particles may enter the corneal epithelium by paracellular or transcellular pathways. Various epithelial cells show different affinity for the molecule, as is exemplified by the relative distribution of chitosan nanoparticles in the conjunctiva and the cornea [70]. The retention of chitosan microspheres at the eye surface may be partly explained by their mucoadhesive properties.

Fig. 4.1. Structure of chitosan

The biodegradation of the polymer is mediated by the hydrolytic actions of lysozyme and other enzymes and results in the formation of oligomers and monomers. The rate of degradation depends on the degree of acetylation in the chitosan molecule (Fig. 4.1), suggesting that modulation of the sustained release of the drug may be possible. De Campos et al. [71] evaluated the potential of cyclosporin-loaded chitosan microspheres in rabbits and concluded that the system has the capability to contact intimately the corneal/conjunctival epithelium. This increases delivery to external ocular tissues without compromising inner ocular structures and with minimum systemic drug exposure, and provides these target tissues with long-term drug levels. Similarly, Genta et al. [72] obtained a 4× increase, compared with an aqueous suspension in the aqueous humour concentration of acyclovir after a single instillation (rabbit eye) of acyclovir-loaded chitosan microspheres. No sign of eye inflammation or discomfort was observed in the rabbits after administration of the microspheres.
Considerable research activity has been focused on the chemical modification of chitin and chitosan. Graft copolymerization with various vinyl monomers may be carried out with several initiator systems [73]. Because of its regular structure and the strong intermolecular and intramolecular hydrogen bonds, the properties of grafted chitosan can be refined to a limited extent. It has been shown that chitosan can be bestowed with improved water solubility and enhanced antibacterial and antioxidant properties by primary derivatisation and graft modification, but there are very few reports concerning the graft copolymerization of chitosan derivatives [73].

Several enzymes including chitinases, chitosanases and lysozyme can effect the degradation of chitosan. In vivo degradability by lysozyme - which is found ubiquitously in the human body (4-13 mg/l in serum and 450-1230 mg/l in tears) [74, 75] - makes chitosan extremely useful to pharmacy and medicine. The action of this enzyme is dependent on factors such as pH, the type of chitin or chitosan, and the preparation method. Davies et al. [76] established that chitosan is most susceptible to lysozyme hydrolysis at pH values ranging between pH 5.2 and 8.0, with an optimum in the acidic range [76, 77]. The natural substrate for lysozyme is the β (1→4) glycosidic linkage, which is found in the negatively charged polysaccharides backbone of certain bacterial cell walls.

Pangburn et al. [78] studied the effect of deacetylation on the susceptibilities of chitin and chitosan to lysozyme and concluded that pure chitin (0% deacetylation) is most susceptible to lysozyme, while pure chitosan (100% deacetylation) cannot be degraded by this enzyme. In 1990, Sashiwa et al. [79] studied the relative rates of degradation of six chitosans with varying degrees of deacetylation (45%, 66%, 70%, 84%, 91%, and 95%), and found that 70% deacetylated chitosan degraded most readily.
4.2. Experimental

4.2.1. Materials and equipment

1-Vinylpyrrolidin-2-one (VP; Sigma-Aldrich) was purified by vacuum distillation; methacrylic acid (MA) was passed through an inhibitor removal column (HQ, Sigma-Aldrich). Solvents (Fischer) and other chemicals [i.e. N-hydroxysuccinimide (NHS); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), chitosan oligosaccharide lactate, the polymerisation initiator 4,4'-azobis(4-cyanovaleric acid) (ACVA; Sigma-Aldrich) and the model drug pilocarpine (Fluka)] were used as received. Phosphate buffer solutions (PBS, pH = 7.4, ionic strength 0.497 M) were prepared as described above (section 2.2.1). The size of the nanoparticles was determined using a Coulter N4 MD sub micron particle size analyser.

The efficiency of the adhesion of polymer minitablets onto hydrated dialysis tubing membrane (cellulose, Visking DTV14000) was evaluated in phosphate buffer solution (pH=7.4) using a Stable Microsystem TATXT Plus Analyzer. The maximum detachment force (MDF) and the corresponding work of adhesion (TWA) were determined at 20 °C; pure chitosan oligosaccharide lactate (CSo) was used as a control.

4.2.2 Synthesis and characterisation of materials

Synthesis of linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers

A Parallel Synthesis Kit (Büchi Syncor) was used for the preparation of the linear cp(VP/MA) copolymers. To aqueous solutions of co-monomers (20 ml; 16.6 %w/w) combined in different VP:MA ratios (see Table 4.3) – ACVA was added (1 %w/w) and solutions were stirred under N₂ at 80 °C, for 8h. The polymers were freeze-dried, finely ground, purified by Soxhlet extraction (THF, 6 h), and finally dried in vacuum (40 °C).
Table 4.3. Feed ratios (w/w) used for the synthesis of poly(vinylpyrrolidone-co-methacrylic acid) linear copolymers, \( \text{cp(VP/MA)} \), and of poly(methacrylic acid), PMA.

<table>
<thead>
<tr>
<th>Material</th>
<th>VP</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>( \text{cp(VP/MA)}_1 )</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>( \text{cp(VP/MA)}_2 )</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>( \text{cp(VP/MA)}_3 )</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>( \text{cp(VP/MA)}_4 )</td>
<td>95</td>
<td>05</td>
</tr>
</tbody>
</table>

Gel permeation chromatography (GPC) was carried out using an Agilent liquid chromatography system (Plaquagel OH 40 8 micrometer column) operating at 40 °C and equipped with a refractive index detector. The mobile phase (flow rate = 1 ml/min) was 10 mmol of phosphate buffer (pH 7.4) and 100 mmol of sodium nitrate. The column was equilibrated for 3.5 h before starting the experiment. Calibration was achieved using a series of polyethylene glycol/polyethylene oxide standards (\( M_p \) 500-5,00,000) at a concentration of 1 g/l (injection volume = 100 µl). The samples were weighed and dissolved in buffer (pH 7.4), and then 5 µl of DMSO (for end of test confirmation) was added in 20 ml of sample solution. The solution was then filtered and injected (100 µl) into the column.

Infrared spectroscopy (FTIR; KBr discs, Unicam RS 1 spectrophotometer, 4 cm\(^{-1}\)) was used as a means of assessing the ratio of the co-monomers within the copolymer structure, by measuring the change in the intensity of the C=O stretching and N-H bending vibrations across the series. An accurate determination of the ratio of the co-monomers within the final product was performed by recording the \(^1\text{H}-\) and \(^{13}\text{C}\) NMR spectra of poly(vinylpyrrolidone-co-methacrylic acid) linear copolymers in D\(_2\)O, using a JEOL spectrometer (operating at 400.13 MHz for \(^1\text{H}\)).

As the hydrophilicity/hydrophobicity balance affects both the adhesive and drug release properties of a material, contact angle goniometry was used as an easily accessible
means of assessing the relative hydrophilicity of the linear copolymers. For copolymers prepared by solution polymerisation, a 3 \% w/w solution was prepared and filtered through a 0.2 \mu m poly(tetrafluoroethylene) filter in order to remove any particulate material. The films were deposited onto poly(methyl methacrylate) substrates by dipping (using a constant speed motor, operating at a dipping speed of 10 mm/sec and a raising speed of 1 mm/s), and then dried at 37 °C for 24 h. The contact angles of the polymers and controls were determined by sessile drop contact angle goniometry in a thermostated cell (20 °C) using a Kruss G10 goniometer interfaced with a video capture apparatus. The contact angles were acquired within 15 s of dispensing a 2 \mu l volume liquid drop, using double distilled water (Aldrich, UK).

General method for the synthesis of hydrogel networks based on poly(vinylpyrrolidone-co-methacrylic acid) copolymers crosslinked via chitosan oligosaccharide

Linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers [cp(VP:MA); prepared cf. Table 4.3] were dissolved with stirring (2 h) in phosphate buffer (pH 7.4). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) and N-hydroxysuccinimide (NHS) were added in different proportions (the volume of PBS and the mass of reagents are given in Tables 4.8 and 4.9), and the reaction mixture was then left stirring for 1 h. The appropriate amount of chitosan oligosaccharide (CSO; cf. Tables 4.8 and 4.9) was dissolved in phosphate buffer (pH 7.4) and added drop wise to the reaction mixture, which was left overnight with stirring. The reaction mixture was then centrifuged (5000 rpm, 10 min), the solid was washed twice with phosphate buffer (pH 7.4) and the product was freeze-dried.
**Synthesis of nanogels based on poly(vinylpyrrolidone-co-methacrylic acid) crosslinked with chitosan oligosaccharide**

Linear poly(vinylpyrrolidone-co-methacrylic acid) copolymer (cp(VP/MA)_1; 0.5 g) was dissolved in PBS (pH 7.4; 50 ml) with stirring (2 h). The solution was filtered, and the appropriate amounts (cf. Table 4.11) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) followed by N-hydroxysuccinimide (NHS) were added. The reaction mixture was allowed to stir for 30 min before a mixture of surfactants (Tween 80 - 15 ml, and Span 80 - 35 ml, dissolved in petroleum ether, boiling fraction 40-60 °C - 300 ml) was added with vigorous stirring. After 30 min, a solution of chitosan oligosaccharide (prepared according to Table 4.11 - in PBS, 2 ml; pH 7.4) was added dropwise, and the emulsion was stirred overnight. Petroleum ether was removed by rotary evaporation, and the surfactants and reaction by-products were extracted with dichloromethane (2×). The aqueous layer was centrifuged (20,000 rpm; 40 min) and the nanogel was freeze-dried.

The particle size was determined at room temperature, using a Coulter N4 MD particle size analyser; for measurements, nanoparticles (0.1 g) were dispersed in distilled water (60 ml).

The swelling properties at pH values of 5.0, 7.4 (physiological) and 9.0 (37 °C) were determined volumetrically (20 mg polymeric powder + 0.5 ml of buffer in a microsyringe) with at least three measurements for each value of the equilibrium degree of swelling - $DS_{eq}$ (%).

$$DS_{eq} = \frac{V_{eq} - V_0}{V_0} \times 100$$

where $V_0$ and $V_{eq}$ are respectively the initial and equilibrium sample volumes.

The efficiency of adhesion was evaluated on wafers prepared from dry, thoroughly ground, materials (30 mg; 2 tonne, 1 min; Ø 8 mm) in phosphate buffer solution (pH = 7.4) using a Stable Microsystem T AXT Plus Analyzer (operating parameters as in section 2.2.2.)
with a test surface of hydrated dialysis tubing membrane (cellulose, Visking DTV14000). Maximum detachment force (MDF) and the corresponding work of adhesion (TWA) were determined at room temperature; pure homopolymer (chitosan oligosaccharide lactate) was used as control.

4.2.3 In vitro drug release studies

Drug loading. The polymeric powder (100 mg) was added to a concentrated solution of pilocarpine hydrochloride (5 ml; 50 %w/w), the mixture was sonicated for 30 min, and then stirred vigorously for 24 h. The loaded nanoparticles were separated by centrifugation (20,000 rpm; 1 h) and freeze-dried. The drug loading capacity was determined by re-suspending the dry polymeric powder (100 mg) in a closed conical flask containing phosphate buffer (pH 7.4; 25 ml) and applying continuous stirring; sampling of the supernatant at regular time intervals (up to 96 h, to ensure the complete release of the drug; the sample volume was replenished with fresh phosphate buffer) allowed the spectrophotometric (215 nm) determination of the released pilocarpine; blank experiments (no drug) confirmed that the polymeric suspension did not absorb significantly in UV.

Drug release. Each in vitro controlled-release experiment was performed on a drug-loaded nanoparticle suspension (100 mg of drug-loaded (10 %) polymeric powder - suspended in 5 ml of phosphate buffer, pH 7.4), prepared and sealed (at the start) in a dialysis membrane. In vitro release profiles under sink conditions (phosphate buffer, pH 7.4; 37 °C) were performed spectrophotometrically (215 nm for pilocarpine) using a dissolution apparatus (Copley DT-70, Erweka Instruments; 500 ml; U.S.P. II apparatus; 25 rpm). At the beginning of each experiment, dialysis bags were introduced (dropped in) at the bottom of the dissolution baths, then the drug release was followed spectrophotometrically, as described above (section 2.2.3).
4.2.4 Biodegradation study

Biodegradability was studied on polymeric wafers (30 mg; 2 tonne, 1 min); each specimen was introduced into a flask (34 °C, thermostated shaker bath) containing PBS (25 ml; pH 7.4) and lysozyme (30 mg; Sigma-Aldrich). Samples (2 ml) were taken after 20, 24, 48, and 120 h. Filtered aliquots (1 ml) were cooled in ice (5 min) and mixed with an alkaline potassium ferricyanide solution (4 ml; prepared from 0.25 g complex salt dissolved in sodium carbonate: 0.5 M, 500 ml). The mixture was placed in a test tube, capped with aluminium foil and incubated in boiling water (100 °C, 15 min). Following rapid cooling (within 5 min) to 25 °C, its optical absorbance was recorded (420 nm, reference: deionised water).

4.2.5 In vivo drug release studies

Drug loaded nanoparticles (125 mg; 10 %w/w pilocarpine hydrochloride) were dispersed in PBS (pH 7.4; 0.5 ml) and administered to rabbits’ eyes (male albino New Zealand, 4.0 - 4.5 kg) by instillation into the lower cul-de-sac (2 drops; 50 μl; equivalent of 1.25 mg pilocarpine hydrochloride). The induced miotic effect was monitored, until disappearance, by timed measurements of the pupillary diameter. Corresponding amounts of aqueous pilocarpine hydrochloride (1.25 mg in 50 μl) were administered dropwise to provide the active control. In separate experiments, nanoparticle suspension without loaded drug provided the blank controls.

Procedures were approved by the Ethical Review Committee at the University of Portsmouth, and all studies were performed in accordance with Home Office regulations for the care and use of laboratory animals.
4.2.6 Corneal tissue penetration by $^{14}$C-radiolabelled nanogels

Radiolabelled nanoparticles np(CSo/cp(VP/MA))\* were prepared according to the method described in section 4.2.3, but $^{14}$C-acrylic acid was used instead of normal methacrylic acid (reagents’ molar ratios are presented in Table 4.11). $^{14}$C-acrylic acid was prepared from commercial $^{14}$C-malonic acid following a published procedure [80]: a mixture of $^{14}$C-malonic acid (0.47 mg, 4.51 mmol, 9.25 MBq) and anhydrous malonic acid (2.079 g, 0.02 mmol) in dry pyridine (8 ml) was refluxed for 2 hours, and concentrated aqueous sulphuric acid was added dropwise to neutralize the cooled (ice bath) reaction mixture. Addition of water (40 ml) dissolved the precipitate that had formed, and the resulting solution was extracted with diethyl ether (3 x 40 ml). The combined ether extracts were dried over magnesium sulphate and after evaporation of the solvent gave the product (colourless oil, 596 mg), which was mixed with acrylic acid (80.00 g) and stored at -8 °C (in separate batches) until use.

The activity of the $^{14}$C-nanoparticles was verified by dissolving samples (100 mg) in sodium hydroxide (1 M; 1 ml), mixing with scintillation cocktail (Ultima Gold-High Flash Point LSC, Perkin Elemer US) and determining their activity using a 1900 TR Packard Bioscience Liquid scintillation counter (LSC).

Rabbit eyes were excised from adult albino rabbits and mounted onto Franz cells (Fig. 4.2). The corneal tissue was exposed to physiological saline at 37.5 °C for 5 minutes, to equilibrate and become wetted. A sample of nanosuspension containing $^{14}$C-radiolabelled nanogels was placed onto the corneal tissue of each eye, and samples (0.5 ml) from the receptor chamber were taken at 15 min intervals for one hour, and then every hour over a 24-hour period. To evaluate the degree of penetration into the corneal tissue, samples were analysed by LSC. Aliquots (0.5 ml) of saline (0.9 %w/w NaCl) were used as blank controls in the LSC measurements.
At the end of the experiment, the corneal tissue was digested (24 h) in aqueous sodium hydroxide (1 M; 1 ml), mixed with scintillation cocktail (3 ml) and analysed by LSC to enumerate radioactivity within the corneal tissue, as a measure of nanoparticle retention.

**Fig. 4.2.** Franz cell (manufactured by Permegear Inc., Bethlehem, PA 18015 USA)

<table>
<thead>
<tr>
<th>cp(VP/MA)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>50:50</td>
<td>50</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>60:40</td>
<td>60</td>
<td>60</td>
<td>75</td>
</tr>
</tbody>
</table>

In order to assess the probability of nanoparticles penetrating through the rabbit’s cornea, as well as the degree of retention of nanoparticles to the ocular surface, the results were compared against those obtained for a crosslinked $^{14}$C-radiolabelled copolymer with a related composition (cp(VP/AA)M_1*), which was applied as a wafer, and acted as a negative control. MBA-crosslinked hydrogel cp(VP/AA)M_1* was prepared by the same method as cp(VP/AA)M_1 (section 2.2.1, Table 2.1) but using radiolabelled $^{14}$C-acrylic acid instead of normal acrylic acid.
4.3. Results and discussion

4.3.1 Synthesis and characterisation of materials

Linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers [cp(VP/MA)] were synthesized by solution copolymerisation; co-monomer ratios used and yields obtained are presented in Table 4.4. The composition of the final product (also presented in Table 4.4, expressed as molar % of MA) was determined by $^1$H-NMR spectroscopy. Characteristically, as the amount of MA in the feed decreases, the proportion of MA units in the product becomes lower than in the feed ratio, reflective of the reactivity ratios of the two co-monomers.

Table 4.4. Feed ratio (w/w), yields and composition (% molar, as determined by $^1$H-NMR) of linear poly(vinylpyrrolidinone-co-methacrylic acid) copolymers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Feed ratio (VP/MA)</th>
<th>MA content (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp(VP/MA)_1</td>
<td>50:50</td>
<td>53.8</td>
<td>75</td>
</tr>
<tr>
<td>cp(VP/MA)_2</td>
<td>60:40</td>
<td>33.8</td>
<td>72</td>
</tr>
<tr>
<td>cp(VP/MA)_3</td>
<td>80:20</td>
<td>8.9</td>
<td>65</td>
</tr>
<tr>
<td>cp(VP/MA)_4</td>
<td>95:05</td>
<td>2.2</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 4.5. GPC calibration results

<table>
<thead>
<tr>
<th>Mp</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>490000</td>
<td>5.8</td>
</tr>
<tr>
<td>217000</td>
<td>6.2</td>
</tr>
<tr>
<td>22100</td>
<td>8.2</td>
</tr>
<tr>
<td>15000</td>
<td>8.4</td>
</tr>
<tr>
<td>2010</td>
<td>9.2</td>
</tr>
<tr>
<td>970</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Mp = peak molecular weight
Molecular weight distributions of the synthesised linear copolymers were determined by gel permeation chromatography (GPC). The calibration curve (Fig. 4.3) was constructed using standard poly(ethyleneglycol)-poly(ethyleneoxide) samples (Sigma-Aldrich; Cat. No. 02393); results of the calibration experiments are presented in Table 4.5.

![Calibration Curve](image)

**Fig. 4.3.** GPC calibration curve (polynomial regression) of poly(ethyleneglycol)-poly(ethyleneoxide) standards (Sigma-Aldrich; Cat. No. 02393).

![Chromatogram](image)

**Fig. 4.4.** Typical GPC chromatogram: linear copolymer cp(VP/MA)_1 [solvent phosphate buffer (pH 7.4, 10 mmol) and sodium nitrate (100 mmol)].
Values of $M_n$ and $M_w$ for each polymer were calculated from the chromatogram by making a series of time / height measurements across the molecular weight distribution range (an example is presented in Fig. 4.4), according to the equations below.

\[ M_n = \frac{\sum h_i}{\sum \frac{h_i}{M_i}} \]

\[ M_w = \frac{\sum h_i M_i}{\sum h_i} \]

where:

$h_i$ = Height of the trace at time $i$.
$M_i$ = Molecular weight of the molecules at time $i$.

Table 4.6. Results of GPC analysis for linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers ($M_n$ = number average molecular weight, $M_w$ = weight average molecular weight, PD = polydispersity index).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_n$</th>
<th>$M_w$</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp(VP/MA)_1</td>
<td>285645</td>
<td>440935</td>
<td>1.54</td>
</tr>
<tr>
<td>cp(VP/MA)_2</td>
<td>168060</td>
<td>384782</td>
<td>2.28</td>
</tr>
<tr>
<td>cp(VP/MA)_3</td>
<td>149560</td>
<td>257102</td>
<td>1.11</td>
</tr>
<tr>
<td>cp(VP/MA)_4</td>
<td>16836</td>
<td>48826</td>
<td>2.90</td>
</tr>
</tbody>
</table>

The results of the GPC measurements for the poly(vinylpyrrolidone-co-methacrylic acid) copolymers are summarized in Table 4.6. All linear copolymers exhibited average molecular weights in the range 48,000 - 440,000 g·mol⁻¹, and a polydispersity index in the range 1.1 to 2.9. Differences in molecular weight distribution profiles may be influenced by the purification procedure: dependent upon solubility, solvent extraction may remove low molecular fractions, alongside un-reacted monomer.

Strong IR bands in the range 800-1000 cm⁻¹, corresponding to the stretching mode of vinyl double bonds [81] present in the monomers, disappeared in the FTIR spectrum of the
copolymers, indicating that polymerisation had taken place and that there was no detectable residual monomer left. The water of hydration attached to the copolymers gives rise to a broad and intense peak at 3378 cm\(^{-1}\). The C-H stretching vibration of the copolymer backbone is manifested through a strong absorption at 2934 cm\(^{-1}\). Bands at 1650–1693 cm\(^{-1}\) correspond to free and associated C=O stretching from monomer units.

As the hydrophilicity/hydrophobicity balance affects both the adhesive and drug release properties of a material, contact angle goniometry was used as a means of assessing the relative hydrophilicity of the linear copolymers (there is a relationship between the contact angles of a liquid drop at the surface of a solid and the surface hydrophilicity/hydrophobicity).

Measurements of contact angles were performed on cp(VP/MA) copolymer films (prepared from a 3 \%w/v solution of copolymer in PBS (pH 7.4) by casting onto poly(methyl methacrylate) substrates, followed by drying overnight at 40 °C) using water as the probe liquid. The contact angles were determined experimentally at 20 °C using the Kruss G10 goniometer software. The presented contact angle data (Table 4.7) are averages from between 8-12 drops on 4-6 independently prepared films. It seems that reducing the proportion of methacrylic acid in the structure effects a decrease in contact angle. As methacrylic acid is hydrophobic in nature, it affects the surface hydrophobicity of the polymer and hence the overall adhesiveness of the copolymer.

Table 4.7. Contact angles, \( \theta \), of linear poly(vinylpyrrolidone-co-methacrylic acid) films.

<table>
<thead>
<tr>
<th>Linear copolymer</th>
<th>( \theta ) °</th>
<th>Standard deviation °</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp(VP/MA)_1</td>
<td>81.6</td>
<td>7.2</td>
</tr>
<tr>
<td>cp(VP/MA)_2</td>
<td>58.2</td>
<td>10.2</td>
</tr>
<tr>
<td>cp(VP/MA)_3</td>
<td>54.1</td>
<td>12.8</td>
</tr>
<tr>
<td>cp(VP/MA)_4</td>
<td>35.1</td>
<td>9.5</td>
</tr>
</tbody>
</table>
In order to create three-dimensional networks appropriate for drug loading and that are also biodegradable, linear poly(vinylpyrrolidone-co-methacrylic acid) copolymers were further crosslinked with chitosan oligosaccharicidic chains (Fig. 4.5).

\[
\text{RCOOH} + \text{EDAC} + \text{CH}_3\text{CH}_2\text{N}(:\text{N})(\text{CH}_2)_3\text{N}(:\text{N})\text{CH}_3 \rightarrow \text{RCOONHR'} + \text{H}_2\text{NR}'
\]

Fig. 4.6. Coupling between carboxyl and amino groups mediated by EDAC [92].

The crosslinking reaction employed makes use of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), which acts as a reaction mediator; the steps of the crosslinking reaction mediated by carbodiimide are depicted in Fig. 4.6, where:

\[ R = \text{linear poly(vinylpyrrolidone-co-methacrylic acid) copolymer chain} \]
\[ R' = \text{chitosan oligosaccharide chain} \]

The linear copolymer \( \text{cp(VP/MA)}_1 \) - which is the least hydrophilic (Table 4.7), and which was obtained with the highest yield (by using a feed ratio of VP/MA of 50/50) - was selected for preliminary studies that were performed in order to optimise the crosslinking reaction conditions. Optimisation experiments involved solvent-phase crosslinking of linear copolymer \( \text{cp(VP/MA)}_1 \) with chitosan oligosaccharide lactate (CSo) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) as a reaction mediator; the presence of \( N \)-hydroxysuccinimide (NHS), acting as an activator, was tested in order to maximize the yield of the crosslinking reaction. The general method employed for the synthesis of hydrogel networks was described in section 4.2.2, and the mass of reagents used, and yields obtained, are presented in Table 4.8.

Table 4.8. Mass of reagents used in optimisation experiments involving crosslinking of \( \text{cp(VP/MA)}_1 \) with CSo, and yields obtained for the chitosan-crosslinked hydrogels \( [\text{CSo/cp(VP/MA)}_1] \).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>( \text{cp(VP/MA)}_1 ) (g)</th>
<th>EDAC (g)</th>
<th>NHS (g)</th>
<th>CSo (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.1</td>
<td>0.10</td>
<td>0.03</td>
<td>0.197</td>
<td>21</td>
</tr>
<tr>
<td>B</td>
<td>0.1</td>
<td>0.10</td>
<td>0.03</td>
<td>0.098</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>0.1</td>
<td>0.05</td>
<td>0.03</td>
<td>0.197</td>
<td>50</td>
</tr>
<tr>
<td>D</td>
<td>0.1</td>
<td>0.10</td>
<td>0.00</td>
<td>0.049</td>
<td>30</td>
</tr>
<tr>
<td>E</td>
<td>0.1</td>
<td>0.10</td>
<td>0.06</td>
<td>0.098</td>
<td>47</td>
</tr>
<tr>
<td>F</td>
<td>0.1</td>
<td>0.10</td>
<td>0.06</td>
<td>0.049</td>
<td>42</td>
</tr>
<tr>
<td>G</td>
<td>0.1</td>
<td>0.05</td>
<td>0.06</td>
<td>0.098</td>
<td>34</td>
</tr>
<tr>
<td>H</td>
<td>0.1</td>
<td>0.10</td>
<td>0.03</td>
<td>0.098</td>
<td>48</td>
</tr>
</tbody>
</table>

\* 0.1 g \( \text{cp(VP/MA)}_1 \) were dissolved in 10 ml PBS, and the general method described at page 172 was followed (10 ml PBS were used for the dissolution of CSo).
Optimisation experiments showed, with some exceptions, that higher yields were obtained when larger amounts of chitosan were used. There is an inverse correlation between yield and the proportion of EDAC to chitosan ratio in the mix; the presence (but not necessarily the amount) of N-hydroxysuccinimide appears to be important.

Following the optimisation experiments, all cp(VP/MA) linear copolymers (Table 4.3) were employed for further crosslinking with chitosan oligosaccharide lactate using the ratios given in Table 4.8, experiment C. The mass of reagents used and the code names for the materials thus obtained are presented in Table 4.9.

The relative composition of the purified materials was assessed in FTIR spectra by estimating intensities of the C=O bands (of the corresponding VP and MA units) and the amide II band (1555 cm⁻¹, N-H bending) - which was used to assess the chitosan ratio in the networks.

Table 4.9. Mass of reagents used for the solution-phase preparation of hydrogel networks based on poly(vinylpyrrolidone-co-methacrylic acid) copolymers crosslinked via chitosan oligosaccharide.

<table>
<thead>
<tr>
<th>Material</th>
<th>cp(VP/MA) (g)</th>
<th>PMA (g)</th>
<th>EDAC (g)</th>
<th>NHS (g)</th>
<th>CSο (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSο/PMA</td>
<td>-</td>
<td>2.0</td>
<td>2.200</td>
<td>1.300</td>
<td>7.890</td>
</tr>
<tr>
<td>CSο/cp(VP/MA)_1</td>
<td>2.0</td>
<td>-</td>
<td>1.100</td>
<td>0.660</td>
<td>3.940</td>
</tr>
<tr>
<td>CSο/cp(VP/MA)_2</td>
<td>2.0</td>
<td>-</td>
<td>0.890</td>
<td>0.530</td>
<td>3.160</td>
</tr>
<tr>
<td>CSο/cp(VP/MA)_3</td>
<td>2.0</td>
<td>-</td>
<td>0.440</td>
<td>0.267</td>
<td>1.570</td>
</tr>
<tr>
<td>CSο/cp(VP/MA)_4</td>
<td>2.0</td>
<td>-</td>
<td>0.110</td>
<td>0.066</td>
<td>0.390</td>
</tr>
</tbody>
</table>

* 2 g cp(VP/MA) were dissolved in 200 ml PBS, and the general method described at page 172 was followed (200 ml PBS were used for the dissolution of CSο).

The swelling behaviour of the CSο/cp(VP/MA) hydrogel networks was examined at 37.5 °C and at several pH values (5.0; 7.4; 9.0) – the method used was described in section 4.2.2. The crosslinked hydrogels exhibited greater degrees of swelling and more rapid
swelling rates at basic pH than at acidic or physiologically relevant pH values (Fig. 4.7). This is consistent with literature reports on crosslinked methacrylate polymers, which confirm that increasing the percentage of crosslinker reduces the rate and degree of swelling and changes the water transport mechanism from Fickian diffusion to anomalous transport [91].

Fig. 4.7. Effect of pH on the equilibrium swelling of CSolPMA and CSolcp(VP/MA) hydrogel networks (means represented; n_{exp} = 3; bars represent standard errors).
The equilibrium swelling and the dynamic swelling behaviour of the synthesised materials were found to be highly dependent upon their chemical composition and upon the environmental conditions (especially pH).

Fig. 4.8. Comparison of equilibrium swelling for materials with different composition, for acidic (5.0), physiological (7.4) and alkaline (9.0) pH values (means represented; \( n_{\text{exp}} = 3 \); bars represent standard errors).
Under acidic conditions, the materials displayed a relatively low degree of swelling, as their carboxylic acid functionalities remained unionised. Under physiologically relevant conditions (pH = 7.4) the materials became more hydrophilic due to the partial ionisation of their carboxyl moieties. Under strongly basic conditions (pH = 9.0), ionisation was complete leading to maximal swelling.

Sensitivity to pH seems to be slightly increased in materials containing a large proportion of VP (Fig. 4.8).

Adhesion measurements on several polymer wafers, prepared from CSo/cp(VP/MA), allowed the determination of the maximum detachment force (MDF) and the corresponding work of adhesion (TWA) (Table 4.10).

Table 4.10. Adhesion data for chitosan-crosslinked hydrogel networks.

<table>
<thead>
<tr>
<th>Material</th>
<th>$MDF_{AV}$ (N)</th>
<th>SD (N)</th>
<th>TWA ($\mu$J)</th>
<th>SD ($\mu$J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSo/PMA</td>
<td>0.36</td>
<td>0.06</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>CSo/cp(VP/MA)$_1$</td>
<td>0.15</td>
<td>0.02</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>CSo/cp(VP/MA)$_2$</td>
<td>0.12</td>
<td>0.02</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>CSo/cp(VP/MA)$_3$</td>
<td>0.17</td>
<td>0.02</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>CSo/cp(VP/MA)$_4$</td>
<td>0.27</td>
<td>0.05</td>
<td>0.13</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$n_{exp} = 5$; $MDF_{AV} =$ average maximum detachment force, $TWA =$ total work of adhesion, $SD =$ standard deviation

The mucoadhesive strength of the polymer samples was sensitive to the relative proportions of MA and chitosan oligosaccharide: the higher the proportion of methacrylic acid the more adhesive the material, consistent with strong interactions between the
carboxylic groups and the test surface. The proportion of chitosan oligosaccharide also influences mucoadhesion, with low degrees of crosslinking promoting stronger interactions.

Nanoparticles of chitosan-crosslinked hydrogels were prepared via an EDAC-mediated reaction performed in emulsion (Tween and Span were used as surfactants) between poly(vinylpyrrolidone-co-methacrylic acid) linear copolymers cp(VP/MA)_1 and chitosan oligosaccharide lactate, as described in section 4.2.2. Different ratios of reagents were used for the preparation of these nanogels (see Table 4.11). Particle sizes were in the nanometer range, with an average of 270 nm (SD = 80). The particles were readily dispersible in aqueous media.

Table 4.11. Mass of reagents used for the preparation of nanogel networks based on poly(vinylpyrrolidone-co-methacrylic acid) copolymers crosslinked via chitosan oligosaccharide, and their 14C-radiolabelled acrylic acid congener.

<table>
<thead>
<tr>
<th>Material</th>
<th>cp(VP/MA) (g)</th>
<th>cp(VP/AA)* (g)</th>
<th>EDAC (g)</th>
<th>NHS (g)</th>
<th>Chitosan (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>np(CSo/cp(VP/MA)_1)_1</td>
<td>0.50</td>
<td>-</td>
<td>0.28</td>
<td>0.17</td>
<td>1.00</td>
</tr>
<tr>
<td>np(CSo/cp(VP/MA)_1)_2</td>
<td>0.50</td>
<td>-</td>
<td>0.28</td>
<td>0.17</td>
<td>0.50</td>
</tr>
<tr>
<td>np(CSo/cp(VP/AA))*</td>
<td>-</td>
<td>0.50</td>
<td>0.33</td>
<td>0.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>

The real composition of the purified nanogels was examined in FTIR spectra by estimating intensities of the C=O bands of the corresponding VP and MA units, and the amide II band (1555 cm\(^{-1}\), broad, N-H bending) - which was used to assess the chitosan ratio in the network (Fig.4.9b). The partial shift of the C=O band, from ~1650 cm\(^{-1}\) in cp(VP/MA) linear copolymers (Fig. 4.9c) to 1693 cm\(^{-1}\) in the final product (Fig. 4.9a) suggests strong interactions between the carboxyl group of methacrylic acid and the amine group of the chitosan oligosaccharide lactate.
Attempts to measure adhesion and swelling data on wafers prepared from dry nanoparticles were largely unsuccesfull because the wafers disintegrated readily in contact with PBS and, as a result, the nanoparticles got easily dispersed.

$^{14}$C-radiolabelled nanoparticles [np(CSo/cp(VP/AA))] have a similar composition to np(CSo/cp(VP/MA)$_1$) (Table 4.1), but were prepared using $^{14}$C-acrylic acid (synthesised from commercially available $^{14}$C-malonic acid, as described in section 4.2.6) instead of methacrylic acid. The method followed for the measurement of their radioactivity is also presented in section 4.2.6.
4.3.2. *In vitro results*

Nanoparticles loaded (10 %w) with the cholinergic antagonist pilocarpine were suspended in phosphate buffer (pH 7.4) and the release of drug was monitored spectrophotometrically (215 nm) over time; a minimum of five experiments were performed in each case, with results presented as averages (Figs. 4.11 and 4.12). The calibration curve obtained for pilocarpine is presented in Fig. 4.10.

![Calibration curve](image1)

**Fig. 4.10. Calibration curve for pilocarpine**

![In-vitro drug release](image2)

**Fig. 4.11. *In vitro* pilocarpine release profile from nanoparticles suspension [np(CSo/cp(VP/MA)_1)_1]; control = aqueous solution containing 10 mg pilocarpine; n_{exp} = 5; standard error bars presented.**
In-vitro drug release (10mg pilocarpine)

Fig. 4.12. *In vitro* pilocarpine release profile from nanoparticles suspension [np(CSo/cp(VP/MA)_1)_2], control = aqueous solution containing 10 mg pilocarpine, means represented; n_{exp} = 5; standard error bars presented.

Blank experiments showed that neither the buffer nor the pure nanoparticulate suspension display any significant absorbance in the UV region of interest (215 nm).

Statistical treatment of the results (ANOVA, two-factors with replication; 5 replicates; p < 0.05) indicated that there is no significant difference between the nanoparticulate formulation [np(CSo/cp(VP/MA)_1)_1] and the control (Fig. 4.11).

Materials with lower crosslinking densities [np(CSo/cp(VP/MA)_1)_2] were found to release the drug at slower rates (Fig. 4.12). Statistical treatment of the results (ANOVA, two-factors with replication; 5 replicates; p < 0.05;) indicated a significant difference between the nanoparticulate formulation [np(CSo/cp(VP/MA)_1)_2] and control solution, and a significant interaction between the type of formulation and time. These results suggest that the more hydrophilic the delivery vehicle the less suitable it is for applications that demand the immobilisation of water-soluble, low molecular weight drugs. Nonetheless, such highly
hydrophilic gels may be useful for the release of active agents that are sparingly soluble in water (e.g. hydrocortisone-acetate), or for that of water-soluble macromolecules (enzymes, proteins, antigens).

4.3.3 Biodegradation study

The biodegradation study was performed on polymeric wafers - prepared from np(CSo/cp(VP/MA)_1) and np(CSo/cp(VP/MA)_1) - using commercially available lysozyme [82, 83]. The adopted method involves the reduction of ferricyanide solution (yellow) in N-acetyl-D-glucosamine (NacGlc) and hot alkali to a colourless product:

\[ \text{Fe(CN)}_6^{3-} + e^- \rightarrow \text{Fe(CN)}_6^{4-} \]

The greater the concentration of NAcGlc, the lower the recorded optical absorption (Fig. 4.13). Calibration plots were constructed from absorbance (420 nm) measurements of a series of standard solutions.

![calibration curve for N-acetyl-D-glucosamine](image)

*Fig. 4.13. The calibration plot obtained for N-acetyl-D-glucosamine.*
Fig. 4.14. Plots of concentration of free chitosan chains detected in solution over time (means represented; n_exp = 3; standard error bars).

The concentration of lysozyme employed in the biodegradation study was chosen to correspond to the concentration in human serum (lysozyme : substrate = 0.6 : 1) [78]. Results (Fig. 4.14) show that the proportion of chitosan in the structure influences biodegradability greatly: higher proportions of chitosan (higher crosslinking densities) may prevent the
enzyme from accessing its site of action. The data are consistent with a continuous biodegradation process, which becomes faster with decreasing crosslinking density. For a higher proportion of chitosan (i.e. in np(CSo/cp(VP/MA)_1)_1), the increase in the rate of biodegradation is very rapid over time, suggesting that the diffusion of the enzyme into the network could represent the rate-limiting step.

4.3.4 In vivo experiments

Readily accessible New Zealand albino rabbits, the ocular model of choice [84], were employed for in vivo studies (two animals; 48 hours rest period between experiments; more details in section 4.2.5). As a positive control, an aqueous solution of pilocarpine hydrochloride was administered (50 µl, containing 1.25 mg active; 2 drops – the same amount as in the nanoparticulate suspension - freshly prepared using np(CSo/cp(VP/MA)_1)_2). The application of drug-free polymeric suspension (blank controls) confirmed that the nanoparticles did not contribute to the observed miotic effect.

The variation in pupillary diameter was monitored over time using a standard pupillary diameter gauge. The presented values (Fig. 4.15) are averages from six experiments. The polymeric suspension containing pilocarpine hydrochloride induced a relatively rapid decrease in pupillary diameter, which was followed by a much slower "pupillary diameter increase" stage. The end of each experiment was signalled by the pupillary diameter returning to its original size.

In all observations (6 experiments) the polymeric suspension containing pilocarpine hydrochloride induced a more pronounced miotic effect than the free drug in a conventional solution. Statistical treatment of the results (ANOVA, two-factors with replication; 6 replicates; p < 0.05;) indicated a significant difference between the nanoparticulate
formulation \([\text{np(CSo/cp(VP/MA)}_1\_2]\) and control solution, and a significant interaction between type of formulation \([\text{np(CSo/cp(VP/MA)}_1\_2]\) and time.

![In-vivo drug release (1.25 mg pilocarpine)](image)

Fig. 4.15. Miotic response in rabbits to instillation of pilocarpine hydrochloride: in solution (●), and loaded in a nanoparticle \([\text{np(CSo/cp(VP/MA)}_1\_2]\) suspension (■), control - aqueous solution containing 1.25 mg pilocarpine; \(n_{\text{exp}} = 6\), means and standard error bars represented.

The results revealed that materials with reduced hydrophilicity, which have a limited swelling capacity, yield more prolonged release profiles. The concentration of methacrylic acid (more hydrophobic than acrylic acid) and the degree of crosslinking are probably the key factors that control pilocarpine release from this type of nanoparticulate system.

In accord with previous studies involving non-ionic surfactants [85], the suspension was found to be very well tolerated by animals.
4.3.5 Corneal tissue penetration by $^{14}$C-radiolabelled nanogels

It has been reported that poly(alkylcyanoacrylate) nanoparticles damage the corneal epithelium by disrupting the cell membrane [86], and previous microscopy studies indicated possible corneal penetration by chitosan nanoparticles [71].

In this work, an in vitro experiment was designed to examine the likeliness of the transport of the chitosan-crosslinked poly(vinylpyrrolidone-co-methacrylic acid) nanoparticles through the rabbit's corneal membrane.

The $^{14}$C-radiolabelled nanoparticles (np(CSo/cp(VP/AA)) - prepared as described in section 4.2.6) - were dispersed in 0.9 %w/w NaCl aqueous solution. The size of the nanogels and the radioactivity of the nanosuspension were highly reproducible (at around 1786 DPM). The radioactivity detected in the samples taken from the receptor chamber of the Franz cell following treatment with the nanosuspension np(CSo/cp(VP/AA)) is summarized in Table 4.12.

Table 4.12. Results of in vitro experiments on diffusion of radiolabelled polymers through the rabbit cornea.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>%activity detected (DPM)</th>
<th>%activity detected (DPM)</th>
<th>Time (hr)</th>
<th>%activity detected (DPM)</th>
<th>%activity detected (DPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>np(CSo/cp(VP/AA))*</td>
<td>Control</td>
<td></td>
<td>np(CSo/cp(VP/AA))*</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>2.51</td>
<td>0.038</td>
</tr>
<tr>
<td>0.15</td>
<td>1.06</td>
<td>0.010</td>
<td>12</td>
<td>2.68</td>
<td>0.039</td>
</tr>
<tr>
<td>0.30</td>
<td>1.11</td>
<td>0.015</td>
<td>13</td>
<td>3.02</td>
<td>0.040</td>
</tr>
<tr>
<td>0.45</td>
<td>1.45</td>
<td>0.018</td>
<td>14</td>
<td>3.35</td>
<td>0.042</td>
</tr>
<tr>
<td>1</td>
<td>1.56</td>
<td>0.019</td>
<td>15</td>
<td>3.52</td>
<td>0.043</td>
</tr>
<tr>
<td>2</td>
<td>1.62</td>
<td>0.020</td>
<td>16</td>
<td>3.75</td>
<td>0.045</td>
</tr>
<tr>
<td>3</td>
<td>1.67</td>
<td>0.022</td>
<td>17</td>
<td>3.97</td>
<td>0.046</td>
</tr>
<tr>
<td>4</td>
<td>1.79</td>
<td>0.025</td>
<td>18</td>
<td>4.00</td>
<td>0.048</td>
</tr>
<tr>
<td>5</td>
<td>1.84</td>
<td>0.026</td>
<td>19</td>
<td>4.25</td>
<td>0.049</td>
</tr>
<tr>
<td>6</td>
<td>1.90</td>
<td>0.028</td>
<td>20</td>
<td>4.70</td>
<td>0.050</td>
</tr>
<tr>
<td>7</td>
<td>1.95</td>
<td>0.029</td>
<td>21</td>
<td>4.75</td>
<td>0.052</td>
</tr>
<tr>
<td>8</td>
<td>2.01</td>
<td>0.030</td>
<td>22</td>
<td>4.81</td>
<td>0.054</td>
</tr>
<tr>
<td>9</td>
<td>2.12</td>
<td>0.032</td>
<td>23</td>
<td>4.81</td>
<td>0.055</td>
</tr>
<tr>
<td>10</td>
<td>2.35</td>
<td>0.035</td>
<td>24</td>
<td>4.81</td>
<td>0.055</td>
</tr>
</tbody>
</table>

DPM = decaying per minute; control = $^{14}$C-radiolabelled cp(VP/AA)M_1*. $n_{exp}$=4, SD < 15%.
The cp(VP/AA)M_1* control appears to have released through the corneal membrane the least amount of radiolabel, irrespective of the length of the treatment period. Statistical treatment of the data (ANOVA, two-factor, without replication) indicated that there is a significant difference between the nanoparticulate formulation [np(CSo/cp(VP/AA))*] and the cp(VP/AA)M_1* control, suggesting that nanoparticles are permeating through the corneal membrane. No significant interaction between time and type of formulation has been found.

![Retention of radiolabel in corneal tissue in-vitro](image)

Fig. 4.16. Percentage of $^{14}$C-activity (LSC) detected in corneal tissue following digestion (sodium hydroxide, 1 M) of the rabbit cornea treated with nanoparticulate formulation [np(CSo/cp(VP/AA))*], and rabbit cornea treated with polymer wafer [cp(VP/AA)M_1*] as control. ($n_{exp}$ =4; means and standard error bars represented)

In order to determine the retention of polymers into the ocular tissue, the corneal membrane was analyzed at the end of the experiment, and Fig. 4.16 summarizes the results of this tissue absorption study. The LSC data showed that there is a significant difference
between the nanosuspension-induced levels of radiolabel absorption into the ocular tissues and that associated with the control; the activity ratio found inside the corneal tissue was 27% of the initially administered dose, much higher than that in the control (only 5%). This indicates a much higher degree of retention at the ocular surface level in the case of the nanoparticles, and could potentially translate into a significant increase in the bioavailability of any active that is delivered using this type of formulation.

4.4 Conclusions

Aimed towards ophthalmic applications, nanoparticles (100-300 nm) of poly(vinylpyrrolidinone-co-methacrylic acid) incorporating short polysaccharidic chains as crosslinks have been synthesised and have been formulated with pilocarpine hydrochloride for delivery through an aqueous medium. The materials possess good bioadhesive properties, which facilitate their retention on the ocular surface. Release experiments have demonstrated that some of these nanoparticles are capable of effecting the controlled release of pilocarpine hydrochloride. In vivo experiments have shown that the nanoparticles are tolerated well by the eye.

To study the in vitro transport of the nanoparticles through the rabbit cornea, $^{14}$C-radiolabelled congeners, [np(CSo/cp(VP/AA))]$, have been synthesised using $^{14}$C-labelled acrylic acid. Results have indicated partial diffusion of the radiolabel through the corneal membrane, suggesting possible corneal penetration by the nanoparticles.

Also, the activity ratio found inside the corneal tissue is 5 x higher for nanoparticles than for the control wafer. This indicates a higher degree of retention at the ocular surface level, which could potentially translate into a significant increase in the bioavailability of any active delivered using this type of nanoparticulate formulation.
4.5. References


Chapter 5 - General discussion and conclusions

Conventional topical ocular treatments have major drawbacks including poor ocular bioavailability (i.e. less than 5% of the administered active is absorbed or becomes available at the site of physiological activity), pulsed drug entry, systemic exposure, and, generally, a low effectiveness for intraocular drug delivery. Therapeutic advances in this field are hindered by the difficulties of increasing bioavailability and of delivering actives to the site of action, especially in the treatment of the posterior of the eye; current research on polymeric delivery may address those issues. Certain polymers are already used as carriers or sustained release vehicles, while 'smart' hydrogels — that react to disease-specific environmental triggers and/or chemical signals to effect drug release — are emerging as components of a new generation of therapeutics (Sections 1.1 and 1.5).

An ideal polymeric drug carrier should have a loading capacity that can ensure therapeutic doses will be able to penetrate to — and/or reside at — the desired site of action, and release the active in a controlled manner. The carrier should also be non-toxic, biocompatible and biodegradable — the latter especially for intraocular administration. For most ophthalmic applications the carrier should not impede vision, since tolerability and acceptance by the patient are critical. The carrier's mucoadhesive properties are very important when surface eye diseases are targeted, while for the treatment of intraocular ailments it must be able to enhance corneal drug penetration.

Efficient drug delivery to the eye still represents a major challenge, despite the considerable research effort dedicated to it in the last decade. Previous studies attempted either to increase the bioavailability of ophthalmic drugs by making use of methods that aim
to improve the corneal penetration of the active and to delay its elimination from the eye, or to achieve controlled release of the drug by using various types of ophthalmic inserts.

This project is directed towards the preparation and evaluation of novel polymeric vehicles that can effect the controlled release of ocular drugs, and it aims to combine recent advances in mucoadhesion and polymer-based drug delivery. Previous work at the University of Portsmouth has established the great promise of polycarbophil and chitosan as mucoadhesives and it is envisaged that, based on these types of polymeric materials, ocular wafers or a nanoparticulate formulation could be developed.

In this work, a number of hydrogel-type polymeric materials - based mainly on polymers with regulatory approval, such as polysaccharides (chitosan), polyacrylates (acrylic acid, methacrylic acid) and poly(vinylpyrrolidone) - have been synthesised and characterised in terms of their chemical and physical structure, using a range of spectroscopic methods, and by swelling and adhesion measurements. The synthesized materials have been formulated as either ophthalmic inserts or nanosuspensions, both of which have been investigated, both in vitro and in vivo, for their ability to act as ophthalmic drug carriers that effect a sustained release and increase the bioavailability of the active. A number of ophthalmic drugs, with either antibiotic or antiglaucoma action (viz. chloramphenicol, norfloxacin, pilocarpine hydrochloride and atropine sulphate), have been employed for this purpose. The degree to which every objective of this project (Section 1.8) has been fulfilled is summarised below, according to each type of material.

Copolymeric hydrogels [cp(VP/MA)M; cp(VP/AA)M] constituted of vinylpyrrolidone and methacrylic or acrylic acid repeat units have been synthesised by radical-induced polymerisation reactions in the presence of N,N'-methylenbisacrylamide crosslinker (Section 2.3.1).
The effects of network composition and drug solubility upon the swelling properties, adhesion behaviour and drug release characteristics have been studied: it has been found that, dependent on composition, \( cp(\text{VP/AA})M \) and \( cp(\text{VP/MA})M \) crosslinked hydrogels exhibit a broad range of swelling and adhesion properties, which can be fine-tuned by altering the relative proportion of each constitutional repeat unit (Section 2.3.2).

*In vitro* drug release studies involving pilocarpine hydrochloride or chloramphenicol have demonstrated that the materials under consideration \( i.e. \ cp(\text{VP/AA})M \) and \( cp(\text{VP/MA})M \) are most appropriate for the delivery of sparingly water-soluble actives \( i.e. \) chloramphenicol or norfloxacin) (Section 2.3.3).

*In vivo* experiments have revealed that the degree of swelling and hydrophobicity of the matrix are key factors in effecting controlled drug release, and have confirmed the potential of \( cp(\text{VP/MA})M \)-based inserts for the ocular delivery of pilocarpine hydrochloride (Section 2.3.4).

A series of hybrid polymeric hydrogels \( \text{hpn(CSfNIPAM); hpn(CSfHEMA)} \), prepared by the reaction of acrylic acid-functionalised chitosan with either \( N \)-isopropylacrylamide or 2-hydroxyethyl methacrylate monomers, have also been synthesised, pressed into minitablets and investigated for their capacity to act as controlled release vehicles for ophthalmic drug delivery (Sections 3.3.1 to 3.3.3).

For comparison, inter-polymeric complex analogues – \( \text{ic(CS/PEMA); ic(CS/PNIPAM)} \) synthesised using the same monomers and pure, un-functionalised chitosan – have been examined by means of an identical characterisation protocol: the effects of network structure and composition upon the swelling properties, adhesion behaviour and drug release characteristics have been investigated (Sections 3.3.1 to 3.3.3).

Comparative *in vitro* studies employing chloramphenicol, atropine sulphate, norfloxacin or pilocarpine hydrochloride have informed the selection of drug-specific carrier
compositions [hybrid polymeric hydrogels hpn(CSf/NIPAM); hpn(CSf/HEMA)] for the controlled delivery of these compounds (Section 3.3.4).

*In vivo* (rabbit model) experiments involving the delivery of pilocarpine have indicated that chitosan-based hybrid polymer networks containing 2-hydroxyethyl methacrylate [hpn(CSf/HEMA)] have a high potential for the delivery of this therapeutic agent (Section 3.3.5).

Hydrogel nanoparticles [np(CSo/cp(VP/MA))] have been prepared from linear poly(vinylpyrrolidinone-co-methacrylic acid) [cp(VP/MA)] that was crosslinked via short polysaccharidic chains (CSo). The materials are biodegradable (Section 4.3.3), and possess good bioadhesive properties, which facilitate their retention on the ocular surface (Section 4.3.1).

Pilocarpine hydrochloride-loaded nanoparticles (100-300 nm) of np(CSo/cp(VP/MA)) have been formulated for delivery through an aqueous medium, and drug release experiments have indicated that some of these nanoparticles are capable of sustaining the miotic effect of the active (Sections 4.3.2 and 4.3.4).

$^{14}$C-Radiolabelled nanoparticles [np(CSo/cp(VP/AA))] have been synthesised and used to study *in vitro* the transport of the nanoparticulate formulation through the rabbit cornea. Results have indicated partial diffusion of the radiolabel through the corneal membrane, suggesting possible corneal penetration by the nanoparticles. In addition, the activity ratio found inside the corneal tissue was $5 \times$ higher for the nanoparticles than that for the control wafer. This indicates a much higher degree of retention at the ocular surface level in the case of the nanoparticles, and could translate into a significant increase in the bioavailability of any active that is delivered using this type of formulation (Section 4.3.5).

All the synthesised polymeric hydrogels (both inserts and nanospuspensions) that have been tested *in vivo* (rabbit model) show a very good tolerance by the eye.
The formulation of biodegradable polymers as colloidal systems holds significant promise for ophthalmic drug delivery, and further work should mainly focus onto the improvement of biodegradability and drug loading capacity of np(CSO/cP(VP/MA)). For this purpose, chitosan chains with different molecular weight and different degrees of deacetylation should be investigated. Such colloidal systems would be suitable for poorly water-soluble drugs, and would allow drop-wise administration while maintaining the drug activity at the site of action.

Additionally, surface-modified nanoparticulate carriers could be used to investigate the release of a wider variety of actives. Although several synthetic methods and drug loading techniques are reported to be safe and reproducible, no procedure for the formulation of drug-loaded nanoparticles has yet been standardised. The major developmental issues in the case of nanoparticles include at present formulation stability, particle size uniformity, control of drug release rate, and large-scale manufacture of sterile preparations.

Surface modification of the colloidal drug carrier might facilitate the modulation of carrier/ocular tissue interaction, leading to materials with selective affinity for the corneal tissue. Natural limiting factors, however, are the low concentration of mucins on the ocular tissue and the high mucin turnover rate.