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FORMULATION AND EVALUATION OF THERAPEUTIC SOFT CONTACT LENSES FOR CONTROLLED DELIVERY OF TRIAMCINOLONE ACETONIDE

IZDELAVA IN VREDNOTENJE TERAPEVTSKIH MEHKIH KONTAKTNIH LEČ ZA NADZOROVANO DOSTAVO TRIAMCINOLON ACETONIDA

Ljubljana, 2011

I have carried out my diploma work at the department of Pharmacy and Pharmaceutical technology (Farmacia e Tecnoloxía Farmacéutica), Faculty of pharmacy, University of Santiago de Compostela, under the supervision of my comentor prof. dr. Francisco Javier Otero Espinar and home mentorship of asst. prof. dr. Pegi Ahlin Grabnar. SEM photographs were obtained at the Unidad de Microscopía de RIAIDT (Red de Infraestructuras y Apoyo a Investigación y a Desarrollo Tecnológico), Santiago de Compostela.

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Statement:

I declare that I have carried out my diploma work independently under the mentorship of asst. prof. dr. Pegi Ahlin Grabnar and comentorship of prof. dr. Francisco Javier Otero Espinar.

Ljubljana, June 2011

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Abstract

Triamcinolone acetonide (TA) is a corticosteroid drug that could be used for its anti-inflammatory action in cases of significant ocular allergy, uveitis, external eye inflammation related to some infections and postoperative inflammation. Ocular therapeutics are mainly administered in the form of eye drops, which results in very poor bioavailability in aqueous humor and systemic side effects as a result of unproductive absorption. Lately, more attention is brought to therapeutic soft contact lenses capable of controlled drug release and which among other things can lower the systemic absorption and improve bioavailability in aqueous humor.

The objective of this research was to prepare poly(hydroxyethyl methacrylate) (pHEMA) based soft contact lenses for ocular delivery of TA, containing ethylene dimethacrylate (EDMA) cross-linker, different proportions of comonomers (N-vinyl-2-pyrrolidone (NVP) and methacrylic acid (MA)) and different percentage (V/V) of water with thermal or photochemical polymerization. Water uptake and swelling behavior, capacity for drug loading and in vitro release profiles were determined to a total of five different hydrogel compositions and three different porosity types. Drug loading and release were analyzed by UV-VIS spectrophotometry.

First experiments involved only the non-porous hydrogels obtained by thermal polymerization. Drug loading was carried out in physiological saline or phosphate buffer solution (PBS, pH 7,4 – 8,0) of TA. The results showed that if physiological saline solutions were used there were only small differences between the lenses. The results were consistent with the results of equilibrium water content (EWC). In the PBS drug solution due to ionization of COOH groups the EWC increased markedly for lenses containing MA and so did the drug loading. The best results were obtained with lenses containing highest amount (200 mM) of MA – these loaded about 0,667 mg/g of drug. The partition coefficients showed that in all cases a high fraction of drug binds to the polymeric network and that the drug demonstrates the same affinity for all hydrogel types. Loading isotherms for both solutions were derived and it could be appreciated that the binding sites were far from being saturated.

In vitro drug release studies with fully swollen drug loaded lenses were carried out in artificial lachrymal fluid (pH 8) at 37 °C. Hydrogels containing MA released the drug significantly faster than the others. Lenses containing 200 mM of MA released 60% of the

dose in the first 8 h and more than 80% in the first 24 h. Drug release was found to be diffusion controlled and release kinetics was shown to be reproducible in all cases.

Subsequent experiments included hydrogels of different porosities containing 200 mM of MA. As the porosity augmented the drug loading in PBS drug solution improved from 0,635 mg/g with non-porous lenses to 0,789 mg/g with the most porous lenses, containing 40% V/V of water. However, the in vitro release kinetics was unchanged, diffusion-controlled as well.

PHEMA based soft contact lenses copolymerized with EDMA and different comonomers could be a good alternative for ocular drug delivery of this corticosteroid drug, providing controlled and reproducible drug kinetics. Augmenting porosity and drug solubility could improve greatly the loading properties of these systems.

Keywords: ophthalmic drug delivery, therapeutic soft contact lenses, controlled drug release/delivery, triamcinolone acetonide, hydrogels, poly(hydroxyethyl methacrylate), pHEMA, methacrylic acid, N-vinyl-2-pyrrolidone, porous material

Povzetek

Triamcinolon acetonid (TA) je kortikosteroidna zdravilna učinkovina (ZU), ki je zaradi svojega protivnetnega delovanja potencialni terapevtik v primerih hude očesne alergije, uveitisa, vnetja zunanjega očesa povezanega z okužbami in pooperativnega vnetja. Aplikacija očesnih zdravil se ponavadi vrši v obliki kapljic, vendar takšna aplikacija vodi v zelo nizko biološko uporabnost zdravila v prekatni vodici in sistemske neželene učinke. Zadnje čase se vedno več pozornosti posveča terapevtskim mehkim kontaktnim lečam, s katerimi je možna nadzorovana dostava zdravila in med drugim zmanjšajo obseg sistemske absorpcije in izboljšajo biološko uporabnost v prekatni vodici.

V diplomskem delu smo s postopkom termične polimerizacije izdelali poli(hidroksietil metakrilne) (PHEMA) mehke kontaktne leče zamrežene z etilendimetakrilatom, z vsebnostjo dveh komonomerov (N-vinil-2-pirolidon, metakrilna kislina) v različnih koncentracijah. V nekatere serije leč smo za povečanje poroznosti vgradili tudi vodo in uporabili fotokemični postopek polimerizacije. Lečam smo določili sposobnost absorpcije vode, nabrekanje, prepustnost svetlobe in s pomočjo UV-VIS spektrofotometrije vrednotili vsebnost ter sproščanje zdravilne učinkovine.

V prve študije smo vključili zgolj neporozne leče, pripravljene s termičnim postopkom polimerizacije. Leče smo potopili v fiziološko raztopino TA in po 4 dneh merili vsebnost - razlike v vsebnosti so bile zanemarljive in ti rezultati so se skladali z rezultati absorpcije vode oz. vsebnostjo vode v ravnotežju. Ko smo leče potopili v izotonično fosfatno puferno (PBS, pH 7,4-8,0) raztopino ZU, pa je bila vsebnost v lečah, ki so vsebovale metakrilno kislino, bistveno večja. Pri tem pH se v leči tvorijo elektrostatske odbojne sile, ki so posledica ionizacije karboksilnih skupin, kar vodi v večjo nabreklost ter absorpcijo vode in posledično večjo vsebnost ZU. Največjo vsebnost ZU smo dobili z lečami, ki so vsebovale največji delež metakrilne kisline, t.j. 200 mM, in sicer je bila ta 0,667 mg/g. Porazdelitveni koeficienti (Kp) so pokazali, da je bil v vseh primerih velik delež ZU vezan na polimerno mrežo in da je učinkovina izkazovala podobno afiniteto za vse tipe hidrogelov. Adsorpcijske izoterme učinkovine v hidrogel so pokazale, da smo bili daleč od nasičenja vezavnih mest.

S popolnoma nabreklimi lečami, ki so vsebovale ZU, smo nato opravili *in vitro* študije sproščanja. Da bi čimbolje posnemali fiziološke pogoje, smo kot medij za sproščanje izbrali umetno pripravljeno solzno tekočino (pH 8) in vzorce inkubirali pri 37 °C.

Pri lečah, ki so vsebovale metakrilno kislino, je bilo sproščanje bistveno hitrejše, zaradi istih razlogov, omenjenih pri študijah vsebnosti v PBS raztopini. Leče, ki so vsebovale 200 mM metakrilne kisline, so v prvih 8 urah sprostile 60% odmerka in v prvih 24 urah več kot 80%. Kinetika sproščanja je bila predvidljiva, saj je bilo sproščanje nadzorovano z difuzijo ZU iz leč v solzno tekočino, ter ponovljiva.

V naslednje študije smo vključili leče z različno poroznostjo, ki so vsebovale 200 mM metakrilne kisline. S povečanjem poroznosti se poveča tudi vsebnost ZU v lečah – ta je bila 0,635 mg/g pri neporoznih in 0,789 mg/g pri najbolj poroznih lečah, v katere smo vključili 40 vol% vode. Kinetika *in vitro* sproščanja učinkovine, je kljub vsemu ostala nespremenjena.

PHEMA leče, zamrežene z EDMA, v katere vključimo različne komonomere, so torej dober nadomestek kapljic za očesno dostavo te ZU, saj nam zagotavljajo nadzorovano in ponovljivo sproščanje. Leče bi se lahko bistveno izboljšale s povečanjem poroznosti in topnosti ZU.

Ključne besede: oftalmična dostava, terapevstke mehke kontaktne leče, nadzorovano sproščanje, triamcinolon acetonid, hidrogeli, poli(hidroksietil metakrilat), pHEMA, metakrilna kislina, N-vinil-2-pirolidon, porozni materiali

List of abbreviations

TA – triamcinolone acetonide

HEMA – 2-hydroxyethyl methacrylate

EDMA – ethylene dimethacrylate

MA – methacrylic acid

NVP – N-vinyl-2-pyrrolidone

AIBN – 2,2'-azo-bis(isobutyronitrile)

NaCl – sodium chloride salt

PBS – phosphate buffered saline

pH – the negative logarithm of the hydrogen ion concentration

pK_a – the negative logarithm of the dissociation constant

T – temperatura

UV – ultraviolet

UV-VIS – ultraviolet-visible

A_{242nm} – absorbance at 242 nm

SD – standard deviation

K_p – partition coefficient

1 Introduction

1.1 Eyes and ocular drug delivery

Human beings have five basic senses to perceive our surroundings. Vision is our dominant sense. Some 70% of all the sensory receptors in the body are in the eyes, and nearly half of the cerebral cortex is involved in some aspect of visual processing (1). Many ocular affections lead to sight loss if untreated so it is very important that effective drugs and ocular delivery systems for the management of these conditions are available.

In the field of drug delivery the eye is a very accessible target. Unfortunately, its anatomy and protective mechanisms make it a challenging target. Research in industry and academia has been active in the last 30 years looking for ways to better deliver drugs to both the anterior and posterior segments of the eye, to achieve adequate bioavailability for better management of ocular diseases (2). Some advances have already been made. From eye drops and eye gels we have come to investigate more sophisticated ocular systems such as ocular inserts and amongst these therapeutic soft contact lenses.

1.1.1 Ocular physiology

The eyeball is a hollow sphere of about 2.5 cm in diameter, maintaining its shape due to the pressure inside it. Its irregular shape reminds of the Earth globe so it is said to have an equator and two poles, anterior and posterior. The most important feature of the eyes is the lens. It is a flexible transparent structure that focuses the light on to retina precisely. The lens and ciliary zonule divide the whole structure of the eye into two segments. A posterior segment behind the lens includes vitreous humor, retina and optic nerve. Anterior segment, which is divided into anterior and posterior chamber, includes lens, cornea, conjunctiva, aqueous humor, iris and ciliary body.

The wall of the eyeball comprises of three coats or tunics. Outermost coat is made of dense fibroblastic connective tissue, therefore it is known as the fibrous layer. We can distinguish between two regions, sclera and cornea. Sclera is the posterior bulk portion that is seen in the front as the white part of the eye. Its role is to protect and shape the eyeball. On the anterior site a transparent cornea comprising only one sixth of the fibrous coat is set into sclera like a watch glass. Together with lens it refracts light therefore it is the first and the

major component of the ocular optical apparatus. About 70% of the total dioptric power of the human eye is due to the interface between cornea and the air (3).

Cornea is one of the most important structures affecting ocular drug pharmacokinetics. It is an avascular tissue organized in three layers of different polarities: epithelium, stroma and endothelium. Corneal epithelium is of hydrophobic nature since it consists of several cell layers. Adjacent cells are joined by tight junctions that do not permit paracellular substance permeation. Stroma on the other hand is a fully hydrated structure. It constitutes about 90% of the cornea. Lying beneath the stroma there's an endothelium, which is hydrophobic as well but has only one cell layer associated with tight junctions. Because of these features cornea represents an important barrier to many foreign substances including drugs. The schematic of cornea is represented in figure 1.

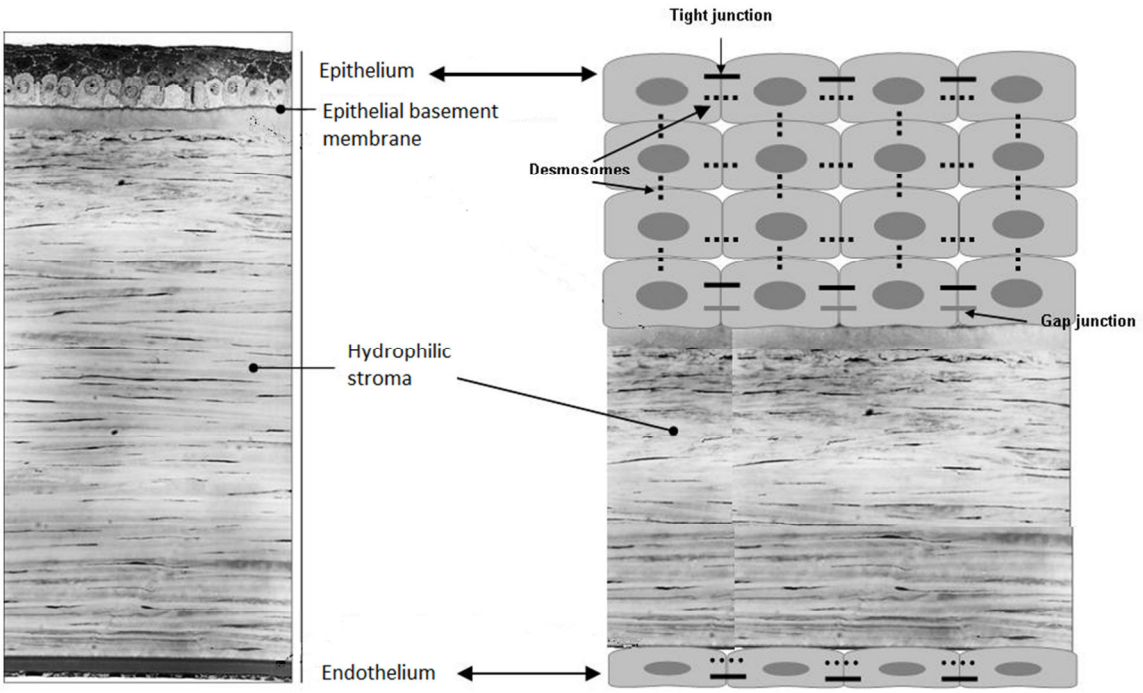


Figure 1: A schematic representation of cornea and its layers. A multilayer epithelium with tight junctions limits the passage of many hydrophilic substances. Hydrophilic stroma on the other hand limits the passage of lipophilic molecules (4).

Middle coat of the eyeball is a vascular coat or uvea that has three regions. Choroid provides nutrition, ciliary body secretes aqueous humor filling the anterior chamber, and iris, the colored part of the eye, acts as an adjustable diaphragm. It varies the size of the pupil to regulate the amount of light entering the eye. Between the blood vessels of iris and aqueous humor lies a selectively permeable membrane named blood-aqueous barrier (BAB), formed by the capillary endothelium and the two layers of ciliary epithelium with tight intercellular junctions. The membrane limits the passage for many substances including large water soluble ions, proteins and other big molecules. Another anatomical protective structure, a blood-retinal barrier (BRB), is found in the innermost eye coat, a nervous coat known as retina, which limits the transport of similar molecules towards the vitreous humor. It is made of retinal pigment epithelium (RPE) and retinal vessel endothelium. BRB and BAB are blood-ocular barriers that isolate the eye from the rest of the body. For this reason systemic administration of ophthalmic drugs is not the best choice, since it means systemic exposure while the drug permeation to intraocular space is very poor.

1.1.2 Accessory extraocular structures

Extraocular accessory structures include eyebrows, extrinsic eye muscles, eyelids, lacrimal apparatus and conjunctiva, last three being of greater importance in ocular pharmacokinetics. Eyelids are thin skin covered folds supported internally by tarsal plate which serve as protection from foreign objects, prevent visual stimuli during our sleep and blink periodically to moisten the eye with tears and sweep the debris. In humans, the average blink rate is 15 to 20 times per minute (5). Tarsal glands found in the tarsal plate secrete an oily substance that lubricates the eyelids and the eye, reduces tear evaporation and prevents eyelids from sticking together (1, 6). Internal surface of the eyelids is lined with conjunctiva. Conjunctiva is a thin transparent mucous membrane that produces lubricating mucus, which protects eyes from drying. It covers the eyelids and folds to the anterior surface of the eyeball, forming a baggy-like structure, a conjunctival sac. The lower conjunctival sac, better known as cul-de-sac is commonly the site of topical administration.

Conjunctiva is highly innervated and therefore very sensitive to pain. It is a highly vascularized tissue which can be seen especially when the blood vessels are dilated in case

of irritated »bloodshot« eyes (6). After the instillation of topical ophthalmic medicine some of the dose absorbs from conjunctiva to local vessels then enters the systemic circulation, hence leading to systemic side effects.

Lacrimal apparatus includes a tear gland and a series of drainage ducts that lead into the nasal cavity. The gland continually secretes tear fluid into the superior part of conjunctival sac. Blinking spreads this fluid over the eye surface where it is drained into the lacrimal sac and enters the nasal cavity via nasolacrimal duct. Nasopharyngeal mucosa is another site of systemic absorption of ophthalmic medicines.

Tear fluid is trilaminar: anteriorly it contains lipids secreted by tarsal glands, which are followed by the dominant aqueous layer from the tear gland. Adherent to the cornea, a posterior layer is a mixture of mucins produced by the conjunctiva. Tears clean and lubricate the eye surface, deliver oxygen and nutrients and also prevent infection by means of bactericidal enzymes, lysozymes. Tear film volume is 7 microliters but can be expanded momentarily to a maximum of 20 microliters. Its turnover rate is some 1,2 µl per minute, which means that in 6 min the entire tear film is renewed.

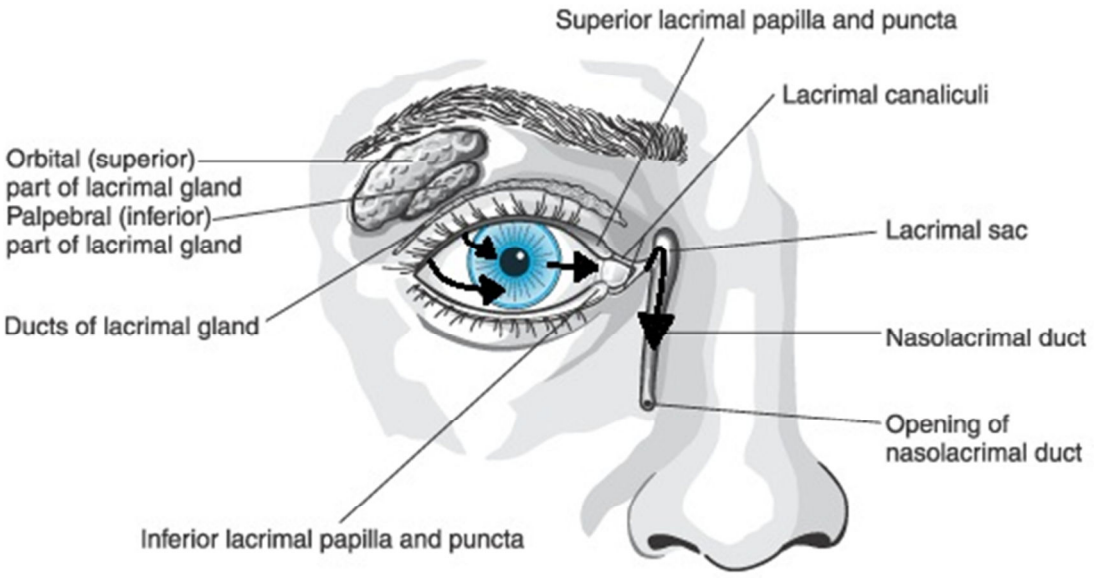


Figure 2: Lachrymal apparatus scheme. Blinking spreads the tears over the eye's surface as shown by arrows (1).

1.1.3 Ophthalmic drug delivery

The eye is a relatively isolated organ owed to the blood-ocular barriers that limit the passage of drugs from systemic circulation to intraocular parts. A best way to address ocular pathologies is thus by local drug delivery. Ophthalmic preparations have several advantages over systemic ones: they bypass the first-pass metabolism therefore lower doses are needed, they cause less systemic adverse effects and because of the direct application the onset of action is faster.

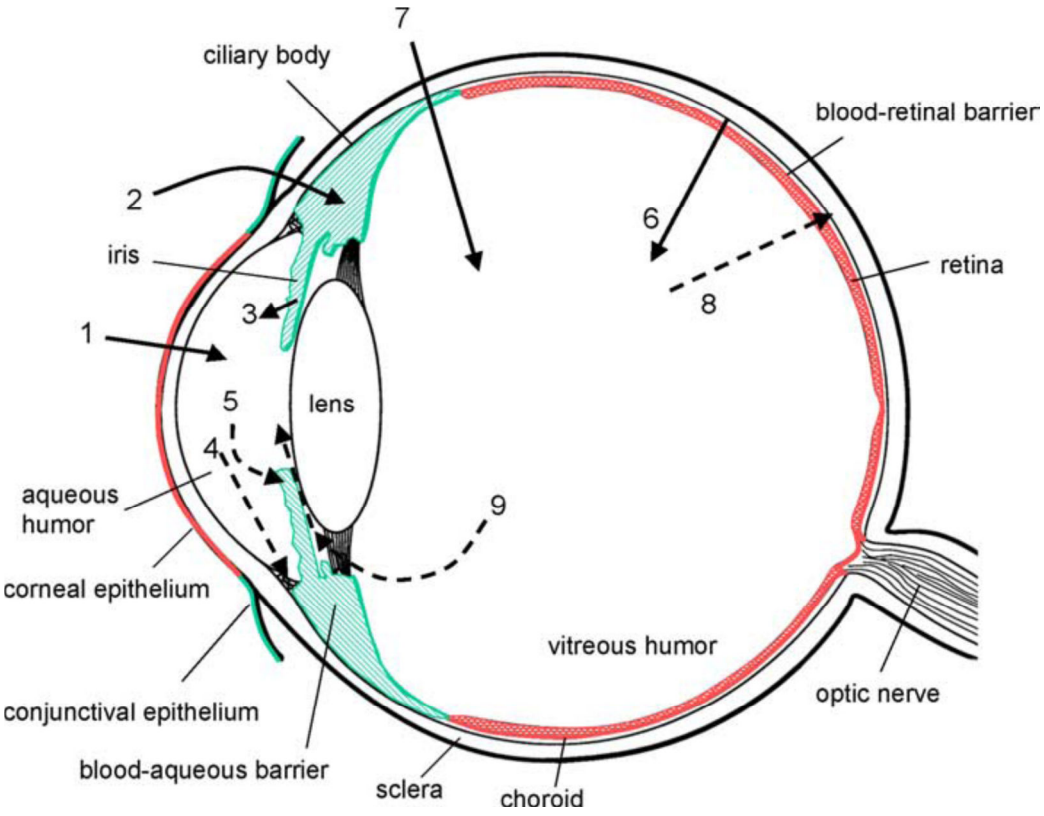


Figure 3: Representation of possible ophthalmic drug delivery pathways (→) and biological eye barriers that lower drug bioavailability in aqueous or vitreous humor. Tight barriers are represented in red, other barriers in green. Elimination pathway is designated by dotted arrow. [1] Topical application, absorption through cornea. [2] Conjunctival and scleral route appropriate for large and hydrophilic molecules. [3] Diffusion through iris blood vessels after systemic administration of small molecule. [4] and [5] Elimination through iris vessels and ciliary body. [6] Very limited diffusion across RPE after systemic absorption. [7] Administration by intravitreal injection, invasive procedure that can cause damage (7).

In clinical practice different routes of ocular drug delivery are possible, depending on the target tissue. For the anterior segment pathologies like inflammation, infection, dry eye syndrome and glaucoma topically administered drugs are used. These should normally be administered in cul-de-sac. Common topical formulations are ophthalmic eye drops. Patients can administer these forms by themselves. On the other hand posterior segment is harder to reach. Diseases affecting the posterior eye are the most prevalent causes of visual impairment in the industrial countries (8). Topically applied drugs cannot reach this far therefore systemic administration, intraocular or periocular injections are used (9). Injections are invasive methods and can cause damage and therefore need to be administered under strict medical supervision. Human sclera seems to be a promising alternative to intravitreal route for posterior segment drug delivering, because it contains 70% of water and has an average surface of 17 cm2 with few protein binding sites (2).

1.2 Topical ocular administration

Eye's external surface is a very accessible zone for drug administration. Topical ocular administration is usually accomplished by eye drops. To exercise its effect on ocular tissues the drug must reach the interior of the eye; it therefore needs to be absorbed through the cornea. The rate and extent of absorption are determined by the time the drug remains in the cul-de-sac and precorneal tear film, elimination by nasolacrimal drainage, drug binding to tear proteins, drug metabolism by tear and tissue proteins, and diffusion across the cornea and conjunctiva (5). Eye drops are quickly drained from the ocular surface and the bioavailability after its instillation has been estimated to be less than 5% (10).

1.2.1 Barriers in topical administration

Productive absorption of topically administered ocular drugs is severely limited by some physiological barriers that ensure a proper functioning of the eye. These can be found in the precorneal as well as corneal area. The most important for ophthalmic pharmacokinetics are the following:

- ***Tear film***

  Upon instillation of eye drop this mixes with the tear fluid present. As mentioned above the volume of tear film can be expanded momentarily to about 20 microliters, but soon reduces to its normal volume of 7 microliters. If the average

commercialized eye drop volume is 40 microliters, some part of the dose must be spilled on the cheek or drained to the nasolacrimal duct. Tear turnover furthermore reduces the concentration of the drug in a way that after 5 min there is less than 40% of the drug left and after 15 min less than 5% left.

- ***Cornea***

  On one hand cornea is the site of entry for topical ocular drugs and on the other it's the biggest barrier that prevents this entry. It has a very low permeability due to its trilaminar structure with alternating polarities of each layer. Epithelium and endothelium omit passage to hydrophilic entities because of their lipophilic nature and moreover due to tight junctions that limit paracellular diffusion. And if the molecule is lipophilic enough to pass the endothelium, hydrophilic stroma then omits its passage.

- ***Conjunctiva***

  Another reason for poor bioavailability is unproductive conjunctival absorption. Conjunctiva as mentioned has lots of blood vessels free of tight junctions. It also possesses a relatively large surface area, 5 times the surface of cornea, thus making the loss significant (11).

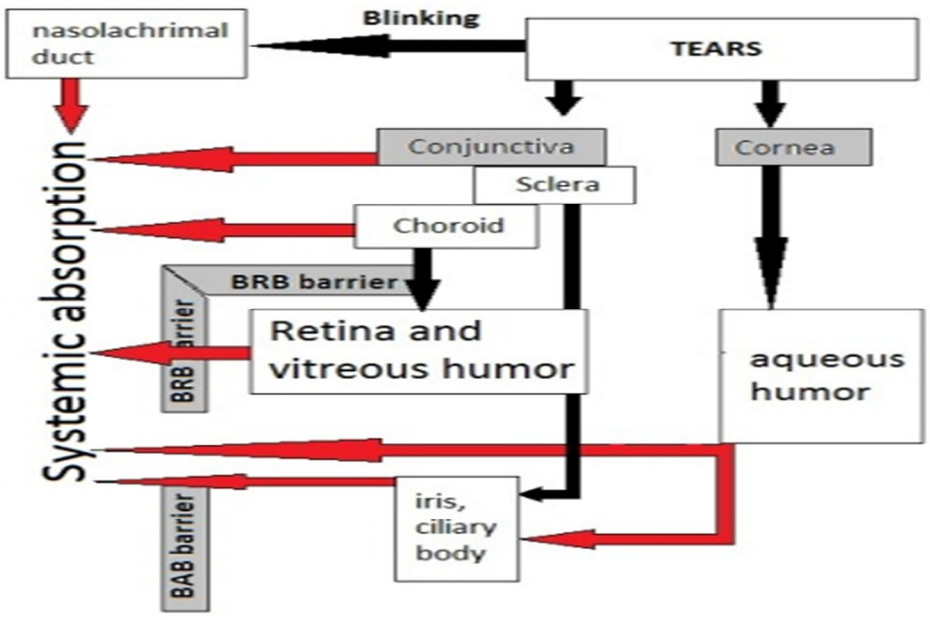


Figure 4: Ocular penetration routes after topical application. The penetration of drug into the intraocular structures is designated by black arrows, the barriers limiting this penetration by grey. The main elimination pathways are indicated by red arrows.

1.2.2 Systemic absorption

Every time we instill an eye drop some unproductive absorption through conjunctiva or nasopharyngeal mucosa occurs, which does not only lead to drug wastage but more importantly to side effects. Considering the high drug concentrations used due to poor corneal permeability and the fact there is no first pass metabolism in liver, quite high blood levels can be reached after a systemic absorption.

In treatment of wide angle glaucoma local eye instillations of β- blockers are used, because they reduce the intraocular pressure (IOP) by reducing aqueous humor production. Systemic absorption produces important side effects mainly on the heart, vasculature, lungs and also kidney. Administration of β- blockers as timolol can cause bradycardias, changes in the QT interval, asthma exacerbations and congestive heart failure as it was reported with timolol. Because they can mask the signs of hypoglycemia and thereby exacerbate it, they should be administered with caution in patients with diabetes mellitus receiving insulin or oral hypoglycemic agents (12).

It's not just side effects; interactions with other medication have to be considered as well. There have been physicians reporting cases of severe bradycardia due to simultaneous administration of verapamil and topical timolol maleate (13).

1.2.3 Formulations with improved corneal penetration

Eye drops are very comfortable for patients to use, but after considering their limitations it became clear that some improvements have to be made in the design of ocular medicines. Researchers focused on prolongation of corneal contact time and enhancement of corneal permeation. Advantages that this brings are less frequent administration, better bioavailability with lower drug concentrations, lower systemic absorption which results in fewer side effects. Unfortunately, many of them also have their down sides for the patient and the manufacturer. Patients might find some forms more difficult to administer or use, some result in blurry vision or gluing of the eyelids. For manufacturers the biggest challenge is sterilization procedure, as all ocular formulations need to be sterile, and provision of sufficient shelf stability, preferably at least 18 months for the formulation to be commercially viable (2). Some advantages and disadvantages of different ocular formulations are gathered in table I.

Table I: Comparison of ophthalmic formulations (2, 6, 13, 14, 15, 16, 17).

Formulation	Advantages	Disadvantages
Eye drops	Ease of administration Unpainful administration Good patient acceptance Low cost	Short residence time Poor bioavailability Sometimes short duration of action Frequent instillation Poor patient compliance Systemic toxicity due to frequent instillation or high concentration
Ophthalmic ointments	Ease of administration Prolonged residence time Less frequent instillation Lack of preservatives Lubricant Low cost	Blurry vision Greasy aspect on the lid margins Slower onset of action Inacurate dosing
In situ forming gels	Ease of administration Less frequent instillation Prolonged residence time	Some blurry vision, less than with ointments
Colloidal systems	Prolonged contact time Controlled release with some Enhanced corneal permeability Improved bioavailability (2-4 times compared to eye drops) Ease of administration More accurate dosing Reduction of systemic absorption	Possible vision interference High costs
Solid ocular forms	Prolonged contact time Most precise controlled release Enhanced corneal permeability Less frequent administration More accurate dosing No preservatives – less sensitivity reactions Longer shelf life Posible combination with other technological approaches (prodrugs, nanoparticle) Reproducibility of release kinetics	Application Sensation of foreign body Possible vision interference Possible loss Necesity of removal
Therapeutic soft contact lenses	No vision interference	

Cornea is a three-layer structure that from outwards begins with a hydrophobic epithelium with tight junctions between adjacent cells. To improve penetration we either have to enhance the lipophilicity by means of prodrug formulations or modify the integrity of

epithelial tight junctions, hence facilitate the paracellular transport. The latter can be achieved by application of electric current or penetration enhancers like chelating agents, surfactants and bile salts, many of which cause local irritation and cytotoxicity. Also formulations of cyclodextrins can be used to this end. Under normal conditions these do not penetrate biological membranes but act as penetration enhancers by assuring constant high concentrations of dissolved drug at the membrane surface (14).

1.2.4 Formulations with improved corneal retention

A more appealing approach to overcome deficits of topical eye solutions seems to be prolongation of corneal contact time and significantly more research has been done in this area. At first, vehicles that should retard drug loss by tear washout have been employed, including ointments and polymeric viscosity improvers. Hydrophilic polymers like hydroxypropylmethylcellulose, sodium carboxymethylcellulose, carbomers, hyaluronic acid, polyvinyl alcohol and polyacrylic acid were used for this purpose. The bioavailability improvement with this formulations was however only moderate, even as the viscosity was increased to a few thousand centipoises (4). Gel formation was the next step in viscosity enhancement, which already permitted only once a day administration. Patient acceptability for these forms is quite low because they might result in blurry vision and sticky eyelids. As far as a large-scale production goes, sterilization of viscous preparations is a major drawback since it has to be done in various steps. Usually the polymer solution is sterilized separately by autoclave or dry heat. Aqueous drug solution is prepared simultaneously and then added to the polymer by sterile filtration (18). The progress in gel technology has brought *in situ* forming gels, which are liquid upon instillation and undergo phase transition in the lachrymal fluid to form a gel. The phase transition can occur due to changes in different factors: it can be owed to a change in pH, temperature or ionic strength. Important advantages of *in situ* over preformed gels are ease of administration and dose reproducibility.

Many hydrophilic polymers used at first as viscous vehicles, which did not have much success, were later discovered to be mucoadhesive thereupon mucoadhesive formulations emerged. Mucoadhesion of hydrophilic polymers is a result of non-covalent bonding to the mucus excreted by conjunctiva. These formulations have been reported to increase the drug bioavailability 2 to 4 times (19). Factors that greatly influence mucoadhesion are polymer hydration or swelling degree, molecular weight, presence of functional groups,

chain flexibility and polymer concentration. Polymer chains and mucus usually interact through hydrogen bonds or hydrophobic interactions and electrostatic interactions in presence of ionizable groups. The latter increase the mucoadhesion significantly.

Amongst mucoadhesive formulations it's worth pointing out the new colloidal systems suitable for administration of poorly water-soluble drugs. They come in liquid form and are very comfortable for the patient. According to their size we distinguish between microspheres and nanospheres or nanocapsules. The drug is dissolved or entrapped in the matrix, encapsulated or adsorbed to the surface. They offer several favorable biological properties such as biodegradability, nontoxicity, biocompatibility and mucoadhesiveness (20). Not only prolongation of contact time but also controlled drug release, enhanced absorption and even endocytosis with nanospheres should improve bioavalability (16).

1.2.5 Controlled ocular drug delivery

Advances in polymeric chemistry brought new solid forms for ocular drug delivery. Mucoadhesive films, collagen shields, rods and inserts are one of the most promising formulations as they are capable of sustained and controlled release. They offer improvement in accurate dosing and bioavalability and reduction of systemic absorption and consequently side effects. Many solid forms unfortunately are still not well accepted by patients due to the difficulties encountered in the application, psychological factors and possible interference with vision (16).

Inserts can be soluble, often referred to as erodible, or insoluble. Only the latter types usually deliver drugs at a controlled, predetermined rate during long period of time (17). The main disadvantage is the necessity of their removal. According to the mode of action insoluble inserts can be subdivided in:

- ***diffusion inserts*** with a central drug reservoir surrounded by a microporous membrane. Lachrymal fluid permeates the device, dissolves the drug creating a concentration gradient due to which the drug is driven out. The membrane controls the rate of drug release.
- ***osmotic inserts*** with an insoluble semi-permeable membrane and a central part. The central part can be one or two compartmental. In two compartmental devices the drug and osmotic agents are placed in two different compartments separated by elastic impermeable membrane. One compartmental device has small drug deposits dispersed in its polymer matrix. In both cases drug/osmotic agent dissolution cause

an elevation in osmotic pressure inside the insert. As a consequence the water enters the formulation causing an elevation in hydrostatic pressure, driving the drug out of the insert.

- ***soft contact lenses (21).***

Soluble ocular drug inserts (SODI) have the advantage to dissolve thus don't need removing. For the same reason the foreign body sensation disappears in only few min after insertion in cul-de-sac. Drug release from these forms occurs when tear fluid penetrates the insert, causing its swelling and gradual dissolution of the polymeric matrix. In the erodible type the dissolution occurs due to chemical or enzymatic hydrolysis of polymeric bonds (17).

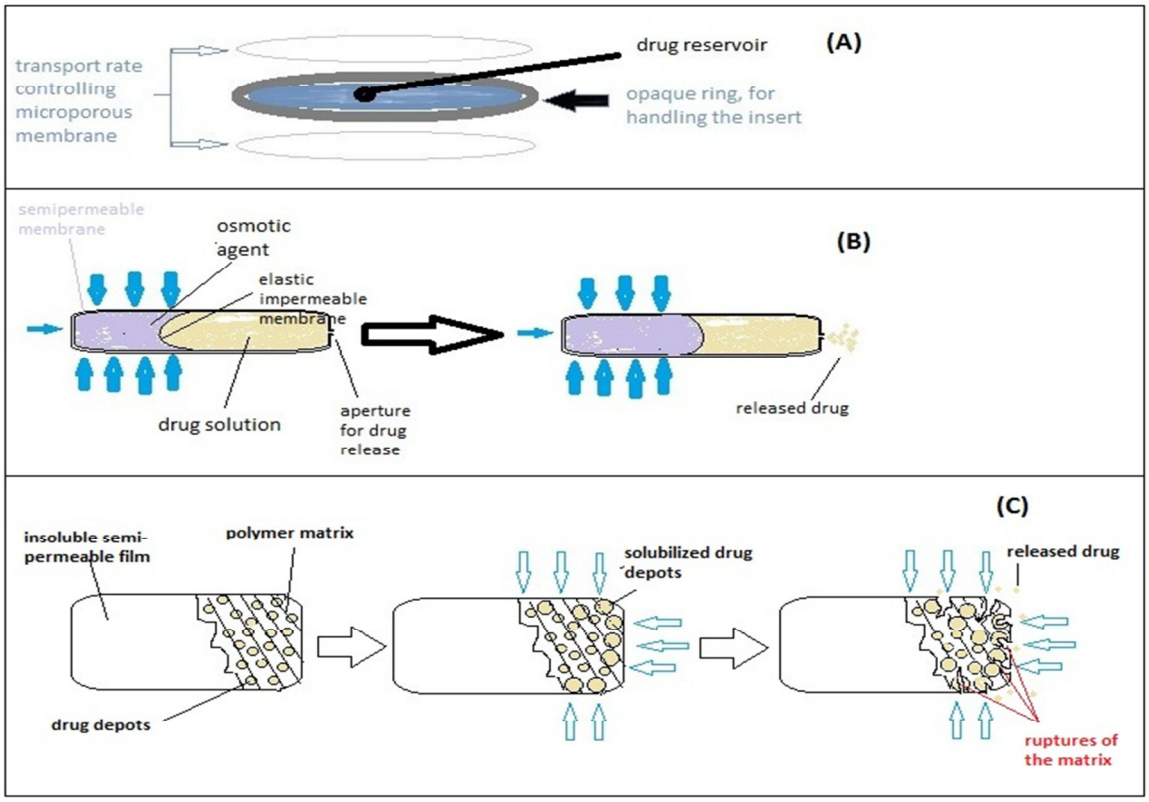


Figure 5: Non-erodible ocular inserts. [A] Diffusional inserts. [B] Osmotic two compartmental system. Expansion of compartment containing osmotic agent after the insert comes in contact with tear fluid causes drug expulsion. [C] Osmotic one compartmental insert. Drug depots dissolve, causing a rise in osmotic pressure that leads to water intake. Polymer matrix cracks due to elevation in hydrostatic pressure thus the drug releases.

1.3 Drug-loaded soft contact lenses

For quite a while now there's an increased interest in developing contact lenses capable of sustained drug release with the objective to improve the efficacy of treatment by the means of higher bioavalability, lower systemic absorption and better dosage regimen compliance. Drug loaded contact lenses could be a great media to correct a vision problem and treat another ocular pathology at the same time and for patients with no vision impairment neutral lenses can be employed. They have the advantage over other types of inserts because they are transparent and hence don't interfere with vision and once managed the technique are quite easy to administer and comfortable to wear. When a drug loaded lens is placed onto the ocular surface a thin tear film gets entrapped between the lens and the cornea leading to drug release, normally driven by diffusion. Turnover of the postlens film is much slower than a normal tear film turnover, which leads to increased precorneal residence time hence better corneal absorption and bioavailability. As a result of the residence time in the postlens tear being about 30 min most of the drug released into it is absorbed in the cornea. A small amount of drug is released to a prelens film as well. It is however several times smaller due to partial dehydration of external surface between interblinking periods (22).

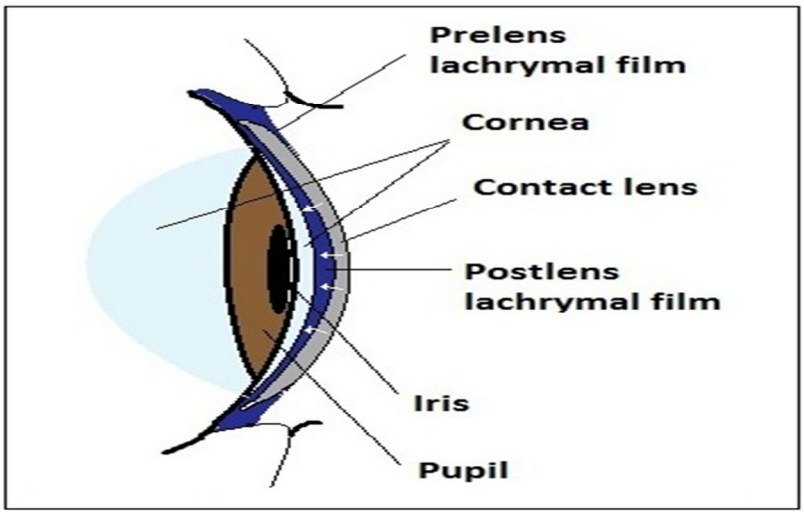


Figure 6: Contact lens inserted in the eye. Drug diffuses from the lens to the postlens lachrymal film as shown by white arrows.

Soft contact lenses are nothing more than hydrogels, a cross-linked network of water soluble monomers. Main property of these materials is swelling in water or other fluids and

retention of these. Their usability as biomaterials is owed to the similarity of their physical properties in swollen state with those of living tissues. This resemblance is based on their water content, soft and rubbery consistency, and low interfacial tension with water or biological fluids (23). Hydrogels are widely used in medicine and other biosciences for tissue engineering, diagnostics, separation techniques and more. They are also of special interest in drug delivery applications, because their structure permits drug loading and subsequent release controlled mainly by diffusion. We can therefore maintain stable local concentration for a prolonged period of time. Nevertheless, hydrogels also have some limitations. Related to drug delivery the quantity and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs. Also the high water content and large pore sizes of most hydrogels often result in relatively rapid drug release, over a few h to a few days (24).

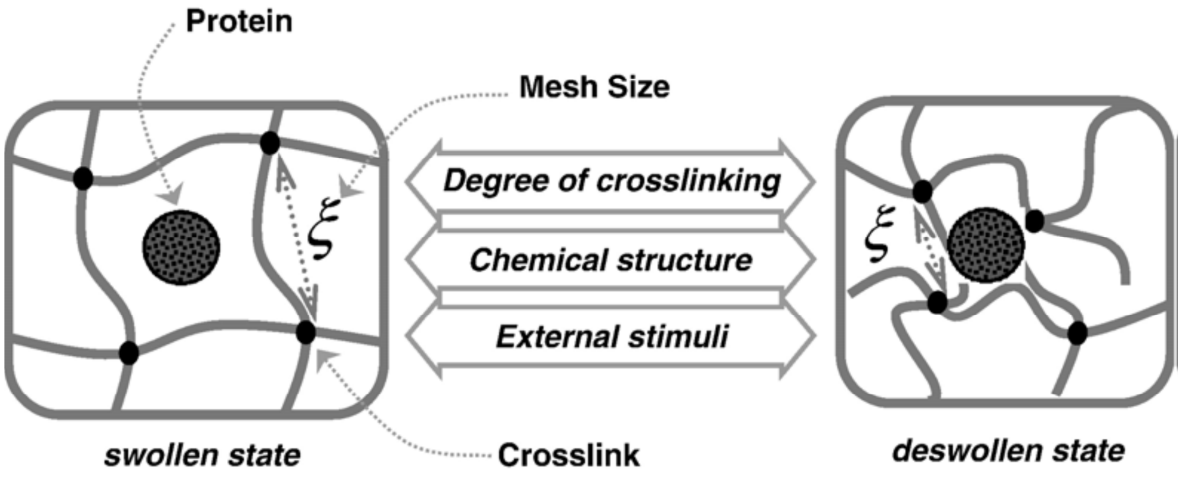


Figure 7: Schematics of polymeric network in dehydrated and swollen state (25), shown on example of hydrogel loaded with model protein.

The basis of many types of soft contact lenses is polyhydroxyethyl methacrylate (pHEMA) hydrogel crosslinked with ethylene glycol dimethacrylate (EGDMA) (26). Another widely used type are silicone-based contact lenses. pHEMA hydrogels are characterized by relatively high water content, thermal and chemical stability, tunable mechanical properties and very important for safe daily wear – oxygen permeability (27, 28). When we immerse pHEMA lens in a drug solution it absorbs the drug distributing it between the polymeric network and its aqueous phase. In pure pHEMA hydrogels drugs incorporate into the network by means of unspecific absorption. The lack of specific interactions limits both the amount loaded and the ability to control the release (29). In order to overcome these limits

different approaches are being investigated. Some employ immobilization of drug by labile bonds, others dispersion of colloidal structures within the lens. Copolymerization with functional monomers, able to interact with the drug through non-covalent bonds, has been shown to be effective provided that their selection is suitable. Therefore, an individulized approach for each drug or family of drugs is necessary. In this way hydrogels for delivery of diclofenac (30), timolol (28), naphazoline (31), oxprenolol, insulin (32) and other drugs have been formulated. The orientation and spatial distribution of functional monomers obtained by this method is random. Molecular imprinting procedures permit the functional monomer molecules to distribute around the drug molecules according to the interactions between them by using a propriate drug as a mold. Lenses specific for one drug can be synthesized in this manner (29).

1.4 Triamcinolone acetonide

Triamcinolone acetonide is a corticosteroid drug effectively used in ocular therapeutics. It is a white or almost white, crystalline powder, practically insoluble in water and sparingly soluble in ethanol. It shows polymorphism (33). It is chemically designated as 9-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione-16,17-acetonide. The empirical formula is $C_{24}H_{31}FO_6$ and molecular weight is 434,50 g/mol. Triamcinolone acetonide is a cyclic acetal derivative of triamcinolone, that can be prepared by stirring a suspension of triamcinolone in acetone in the presence of a trace of perchloric acid (34). It is 10 times more active topically than triamcinolone itself.

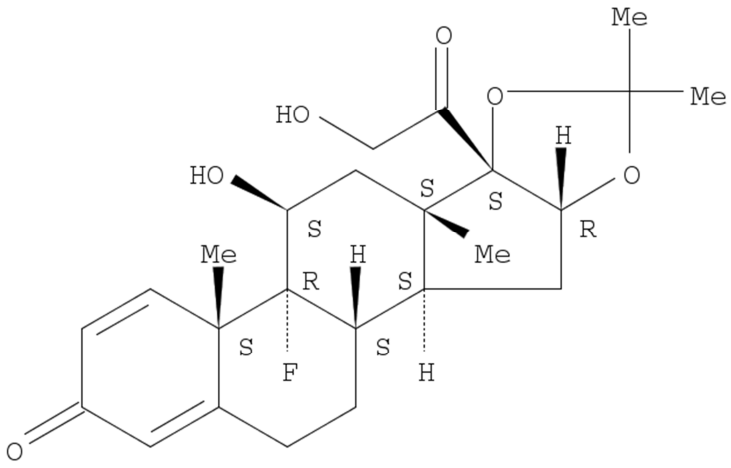


Figure 8: Structural formula of triamcinolone acetonide (35).

Corticosteroids belong to a group of steroid anti-inflammatory drugs. They bind to nuclear GR receptors and modify gene transcription hence protein synthesis. Amongst other things, this leads to inhibition of phospholipase A2 (PLA2) cyclooxygenase (COX) and in blocked production and liberation of cytokines, therefrom the anti-inflammatory action of these substances. Relative potencies of anti-inflammatory action of some corticosteroids are as follows: hydrocortisone 1, prednisone 4, methylprednisolone 5, triamcinolone acetonide 5, fluorocortisone 10, betamethasone 25, and dexamethasone 25 (36). Dexamethasone (MAXIDEX), prednisolone (PRED FORTE), fluorometholone (FML), loteprednol (LOTEMAX), triamcinolone acetonide (KENALOG injection) are used for their anti-inflammatory action in significant ocular allergy, uveitis, external eye inflammation related to some infections and postoperative inflammation. Triamcinolone acetonide is marketed as an injectable suspension (Kenalog®-10, Triesence™, Trivaris) to treat inflammatory and proliferative ocular disorders mainly uveitis, cystoid macular edema and proliferative vitreoretinopathy.

2 Objectives

The objective of this diploma work is to prepare and evaluate pHEMA based soft lenses for ocular delivery of triamcinolone acetonide. We will incorporate EDMA cross-linker and different proportions of comonomers that are normally used for improvement of mechanical and oxygen permeability properties of soft contact lenses. We will prepare five different types of hydrogels by thermal curing using azo-initiator. Afterwards lenses of elevated porosity will be prepared by incorporation of water in the monomer solutions using photochemical polymerization procedure.

Microstructure, absorption of water, swelling and transparency of prepared lenses will be determined. For evaluation of our lenses drug loading and *in vitro* drug release studies will be carried out. Lenses will be submersed in different drug solutions for several days after which the amount of loaded drug will be determined. Drug loaded samples will be submersed in lachrymal fluid to release the drug and the release profiles will be determined. First experiments will include only lenses obtained by thermal curing, to evaluate the effects of hydrogel type and drug loading solution. The best combination of these two factors will be chosen to do further drug loading and in vitro drug release studies on porous lenses in order to evaluate the impact of microstructure on lens performance.

3 Experimental work

3.1 Materials

3.1.1 Reagents for lens synthesis

2-Hydroxyethyl methacrylate (HEMA), Merck Schuchardt OHG, Hohenbrunn, Germany

Ethylene dimethacrylate (EDMA), Sigma-Aldrich Chemie GmbH, Steinheim, Germany

N-vinyl-2-pyrrolidone (NVP), Merck Schuchardt OHG, Hohenbrunn, Germany

Methacrylic acid (MA), Merck Schuchardt OHG, Hohenbrunn, Germany

2,2'-azo-bis(isobutyronitrile), Acros Organics, Geel, Belgium

Irgacure® 2959, BASF Kaisten AG, Kaisten, Germany

Dichlorodimethylsilane, Merck Schuchardt OHG, Hohenbrunn, Germany

Sodium hydroxide pellets, Merck KGaA, Darmstadt, Germany

Hydrochloric acid (37%), Panreac Quimica SA, Barcelona, Spain

Ultrapure water, MilliQ®, Millipore Spain, resistivity ≤ 18MΩcm

3.1.2 Reagents for preparation of media used in pharmacokinetic studies

Triamcinolone acetonide, ≥99,6% purity, Fagron Iberica, S. A. U., Spain

Sodium chloride, Panreac Quimica SA, Barcelona, Spain

Sodium bicarbonate, Bochrom AG, Berlin, Germany

Potassium chloride, Merck KGaA, Darmstadt, Germany

Calcium chloride 2-hydrate, Panreac Quimica SA, Barcelona, Spain

Potassium *di*-hydrogen phosphate, Panreac Quimica SA, Barcelona, Spain

di-Natrium hydrogen phosphate, Merck KGaA, Darmstadt, Germany

Purified water

3.1.3 Materials and devices

Glass plates

Silicone frame

Digital analytical balance, Denver, AA-200

Multipoint magnetic stirrer

UV lamp

Owen Heraeus

Cork borer

Pipette Multipette® Stream, 50 mL

Pipette Biopettes, Labnet, 2-20 µL, 20-200 µL, 100-1000 µL

Diode Array UV-VIS spectrophotometer, Agilent 8453 connected to Hewlett Packard Vectra computer

Quartz Suprasil® cell (cuvette), light path 10.00 mm

pH Meter, MicropH 2001, Crison

Cellulose acetate filters, filter type 0.45µm (Sartorrius Stedim Biotech GmbH, Germany)

Sartolon Polyamid filter, filter type 0.45µm (Sartorrius Stedim Biotech GmbH, Germany)

Glass vials (type I) with chlorobutyl elastomers

Heidolph Inkubator Platform shaker 1000

Digital caliper, Powerfix®Profi+, model Z22855F, Milomex Ltd

3.2 Sample and media preparation

3.2.1 Lens formulation

- ***Nonporous lenses.***

Hydrogels were prepared by radical solution polymerization with thermal initiation. 6 ml of monomer solution were prepared by dissolving the amount of cross-linker (EDMA) necessary to reach concentration of 80 mM and different proportions of functional monomers (NVP, MA) in HEMA. Detailed composition of each series is shown in table II. After stirring for 45 min a thermal azo-initiator AIBN in 10 mM concentration was added. The mixture was stirred until the initiator had completely dissolved and was then injected into the molds. These were made of two glass plates, previously covered with dichloromethylsilane and separated by a silicone frame (≈1 mm thick). The molds were placed in an oven heated to 50 °C for 12 h, then moved to an oven heated to 70 °C and left

there for 24 h to complete the polymerization. After the polymerization step was completed each gel was immersed in boiling water for 15 min to remove any unreacted monomers and to facilitate disc cutting (29). The discs were cut with a cork borer. Before we could use them for further studies they needed to be washed to assure their biocompatibility. They were submersed in 10 mM NaCl solution for 3 days, 10 mM HCl solution for another day and finally in ultrapure water (MilliQ®, Millipore Spain, resistivity ≤ 18 MΩcm) until they were clean. The media were replaced two times a day. Cleanness was verified by recording UV spectra over the range 190 to 800 nm where a complete peak absence was needed. Clean discs were dried at 37 °C to constant mass.

Table II: Monomer mixture composition for fabrication of soft contact lenses.

Series name	Functional monomer	HEMA : EDMA : Funct. monomer (volume ratio)
0	No comonomer	5910 : 90 : 0
MA100	Methacrylic acid, 100mM	5860 : 90 : 50 (MA)
MA200	Methacrylic acid, 200mM	5810 : 90 : 100 (MA)
NVP100	N-vinyl-2-pyrrolidone, 100mM	5846 : 90 : 64 (NVP)
NVP200	N-vinyl-2-pyrrolidone, 200mM	5782 : 90 : 128 (NVP)

- ***Lenses with modified microstructure – porous lenses.***

For the preparation of porous lenses a procedure similar to the one described by Yañez et al. was followed (37). Increase in porosity is due to microphase separation during the polymerization procedure, when water soluble HEMA converts to a non-soluble polymer matrix. The thermo-curing process used for fabrication of previous lenses was replaced by photo-polymerization in order to promote a rapid formation of the hydrogel and prevent segregation of aggregates of insoluble pHEMA that would give rise to a heterogeneous product.

Monomer solutions of the same composition as shown in table II were prepared, this time in larger volumes. After stirring for 45 min, aliquots of each solution were drawn with a pipette and mixed with determined volumes of purified water (table III) - after the addition of photochemical initiator Irgacure® 2959, solutions were immediately injected into the molds. The molds were irradiated with UV-lamp at 366 nm for 40 to 60 min until complete polymerization. The same procedure as described above was used for the elimination of monomer residuals, disc cutting, lens cleaning and drying.

Table III: Proportions of water added to MA100, MA200, NVP100 and NVP200 monomer mixtures to obtain different porosity types.

Porosity type	Monomer solution : water (volume ratio)
0	100:0
20	80:20
40	60:40

Table IV: Lens series prepared by UV-polymerization using different proportions of water-polymer mixture.

Series name	Functional monomer	Water content [vol%]
0_p0; 0_p20; 0_p40	No functional monomer	0; 20; 40
ma100_p0; ma100_p20; ma100_p40	MA 100mM	0; 20; 40
ma200_p0; ma200_p20; ma200_p40	MA 200mM	0; 20; 40
nvp100_p0; nvp100_p20; nvp100_p40	NVP 100mM	0; 20; 40
nvp200_p0; nvp200_p20; nvp200_p40	NVP 200mM	0; 20; 40

3.2.2 Calibration curves

Spectrophotometric methods of analysis were used in drug loading and release experiments. Three different media were used: 0,9% NaCl solution or PBS buffer (pH 7,4 - 8) for drug loading and artificial lachrymal fluid (pH 8) for drug release studies. Calibration curves for TA in three different media were therefore prepared. Absorbances of standard solutions were measured at λ_{max} for TA (242 nm), which was determined by recording UV spectra of water solution in range of 190 – 500 nm (figure 9). Calibration curves, the squares of Pearson coefficients (R^2) and the analysis of variance of the regression model were obtained by linear regression with the help of GraphPad computer program (GraphPad software Inc., USA).

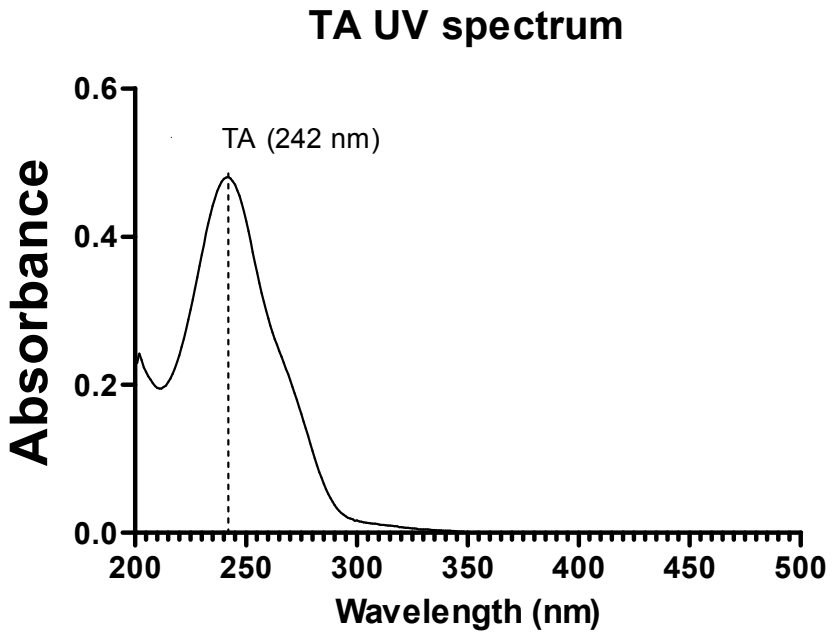


Figure 9: Characteristic UV band for TA - recorded in water solution.

- ***0,9% NaCl solution, physiological saline***

Accurately weighed 9,0 g of NaCl were quantitatively transferred to a 1000 mL volumetric flask. The flask was filled to the mark with purified water and shook to dissolve the salt. Four different ethanol bulk solutions of TA (1,0 mg/ml) were prepared. TA was accurately weighed (0,050 g) and quantitatively transferred to a 50 ml volumetric flask. The flask was filled with absolute alcohol and shook. TA dissolved readily.

According to data available on TA water solubility standard saline solutions with a concentration range from 1 – 20 µg/ml were prepared by dilution of ethanol bulk solutions. Calculated amount of bulk solution was pipetted and mixed with saline solution in a 50 ml volumetric flask, then left to stir on a magnetic stirrer. Absorbance at 242 nm of these solutions was measured with UV-VIS spectrophotometer, Agilent 8453 using a quartz cuvette and 0,9% NaCl as blank solution.

- ***PBS buffers with pH 7,4 and 8,0***

For a PBS buffer solution 16,0 g of NaCl, 0,2 g of KCl, 3,78 g of $Na_2HPO_4 \cdot 12H_2O$ and 0,24 g of KH_2PO_4 were accurately weighed and transferred to a 1000 mL volumetric flask. The flask was shook to dissolve the salts. Buffer pH was adjusted to 7,4 or 8,0 by adding drops of 0,1 M NaOH or HCl solutions while stirring (magnetic stirrer). For preparation of bulk solutions TA was accurately weighed and transferred to a 50 ml volumetric flask, which was then filled with absolute alcohol. After appropriate dissolution with PBS buffer, following the procedure described above standard solutions with concentration range from 1 – 20 µg/ml were obtained and analyzed spectrophotometrically (blank: PBS).

- ***Lachrymal fluid pH 8,0***

To approximate the conditions to the physiological ones artificial lachrymal fluid was chosen as a release media. 6,78 g of NaCl, 2,18 g of $NaHCO_3$, 1,38 g of KCl, 0,084 g $CaCl_2 \cdot 2H_2O$ were accurately weighed to a 1000 ml volumetric flask. The flask was filled with purified water to the mark. It was shook well to completely dissolve the salts. The pH was adjusted to 8,0 with 0,1 M HCl and 0,1 M NaOH solutions.

Concentration range in these studies was from 0,2 – 10,0 µg/ml because the sink conditions have to be assured at all time. The concentration of ethanol bulk solutions was lower than before (0,5 mg/ml) to facilitate pipetting when preparing standard solutions. These were analyzed spectrophometrically (blank: lachrymal fluid).

3.2.3 Preparation of solutions for drug loading

- ***TA in 0,9% NaCl***

2 liters of physiological saline solution were prepared. About a half of it was distributed amongst several flasks where TA was added in excess. Flasks were left in a shaking water bath at 40 °C for two days. After this time samples were taken out of the bath, left to cool at a room temperature and filtered using a Sartolon Polyamid filter type 0,45 μm. Concentrations of the filtrates were determined spectrophotometrically and adjusted to desired values by dilution with the remaining 0,9% NaCl solution using a 500 ml volumetric flask.

- ***TA in PBS buffer with pH 7,4 or 8,0***

2 liters of PBS buffer solution with pH 7,4 or 8,0 were prepared by a previously described procedure. Approximately 1 L of it was transferred to several flasks. TA was added in excess and from there on the above procedure repeats. For dilution of the primary solution the remaining PBS buffer was used.

3.3 Methods

3.3.1 Lens characterization

- ***Microstructure***

SEM images were recorded to observe changes on the surface after incorporating water in the monomer mixture. Dry hydrogels were cut into small pieces that were attached to an aluminium support with a double-sided tape and coated with gold (Thermo VG Scientific POLARON SC7640 sputter coater, UK). Micrographs were taken at amplifications 500x, 1000x using a scanning electron microscope EVO LS15 Zeiss, Germany.

To perform surface analysis 1024x768 grey scale images were stored in TIFF format which allowed a 256 intensity level. The pore size distributions on the hydrogel's surface were estimated using the count-size moduli of the ImagePro-Plus 5.0 software package (Media Cybernetics). An automatic threshold was used to convert grey scale to binary images.

- ***Swelling kinetics and water uptake***

Dry discs, 6 replicates of each hydrogel type, were accurately weighed and dimensions were measured (diameter and thickness). Samples were immersed in 0,9% NaCl or PBS solution. At regular intervals the discs were taken out of the solution and carefully wiped with a tissue. They were weighed and immediately returned to the same liquid. The procedure was repeated until the equilibrium water content (EWC) i.e. constant mass was reached. At the end the final diameter and thickness were annotated once again. Swelling kinetics for each type of lens was calculated as the relative weight gain at time of weighing (equation 1). Equilibrium water content (%EWC) was expressed as final weight gain per gram of dry lens (equation 2).

$$Q(\%) = \frac{(w_t - w_0)}{w_0} \times 100 \qquad \text{(Eq. 1)}$$

Q(%) – swelling in percentage

w_0 – initial weight

w_t – weight at time t

$$\%EWC = \frac{w_\infty - w_0}{w_0} \times 100 \qquad \text{(Eq. 2)}$$

EWC – equilibrium water content

w_∞ – final/equilibrium weight

- ***Optical transparency***

Optical transparency of fully swollen hydrogels was measured at 600 nm. Quartz cuvette was filled with purified water to do a blank measure. Discs were then fixed to the inner side of the cuvette filled with water and transmittance at 600 nm was recorded.

3.3.2 Drug loading and in vitro drug release experiments

- ***Loading studies***

For every lens type 6 dry lenses were accurately weighed. Every lens was placed in its own glass vial that was marked with hydrogel type and lens number. 10 ml of drug solution

were added using a pipette (Multipette). Concentrations of these were determined spectrophotometrically (242 nm) before the experiment started.

Vials were kept in a dark space at a room temperature for 4 days. Every day they were examined to see if any lenses needed detaching from the vial's bottom. After 4 days the final concentration of solution in each vessel was determined spectrophotometrically. The amount of TA loaded by a lens was estimated as a difference between initial and the remaining amount of drug in the solution (equation 3).

$$Q_{load} = (c_0 - c_t) \times V \quad \text{(Eq. 3)}$$

Q_{load} – amount of TA loaded

c_0 – concentration of loading/surrounding solution

c_t – concentration of solution after time t (96 h)

V – volume of surrounding solution

Finally the lenses were then taken out of the vials and lens volume and drug concentration in external medium were used to determine the partition coefficient K. This is defined as the ratio of the drug concentration in the gel and the concentration in the aqueous phase at equilibrium (equation 4). (38)

$$K_p = \frac{c_g}{c_w} = \frac{V_w \times (c_{w,0} - c_{w,t})}{V_g \times c_{w,t}} \quad \text{(Eq. 4)}$$

K_p – partition coefficient

c_g – drug concentration in lens

c_w – drug concentration in external medium

V_w – volume of external medium

V_g – volume of fully hydrated lens

$c_{w,0}$ – initial concentration in external medium

$c_{w,t}$ – final concentration in external medium

- ***In vitro drug release experiments***

Loaded lenses (6 replicates) were rinsed with water to eliminate any TA adsorbed to the surface and then submersed in 5 ml of artificial lachrymal fluid in glass vials. These were closed tight to prevent liquid evaporation and then incubated at 37 °C while shaking in Heidolph Inkubator Platform shaker 1000 (figure 10). At regular intervals 1 ml samples were withdrawn and replaced with the same volume of fresh solution. Sink conditions were kept throughout the experiment. Samples were analyzed spectrophotometrically using a low volume cuvette (242 nm, blank: lachrymal fluid). The amount of drug released was calculated as the amount of TA in solution at the time of sampling added the amount of TA lost by previous samplings (equation 5).

$$Q_{rel} = c_t \times V_{LF} + \sum_{i=1}^{t} c_t \times V_x \qquad \text{(Eq. 5)}$$

Q_{rel} – amount of released drug (µg)

c_t – concentration of media at time of sampling

V_{LF} – volume of lachrymal fluid (5 ml)

V_x – sample volume (1 ml)

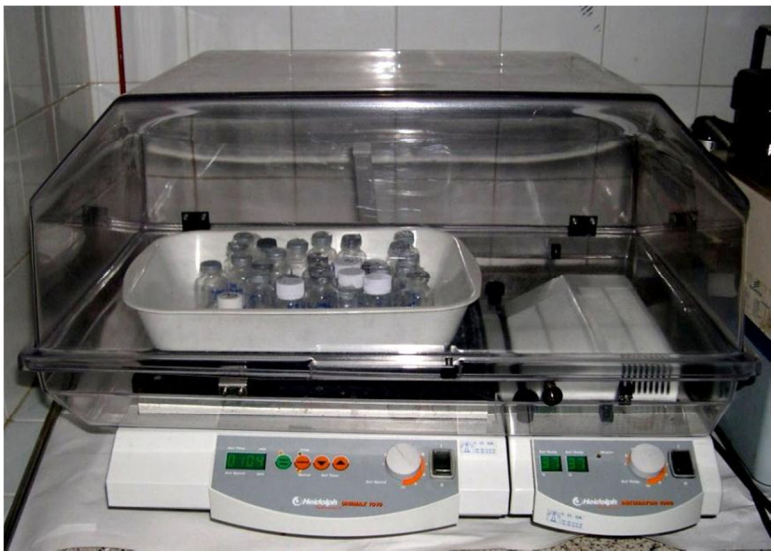


Figure 10: The incubator platform shaker used for drug release.

- ***Computer analysis of results***

For derivation of loading isotherms and drug release profiles Microsoft Office Excel and GraphPad Prism 5.0 were used. Release profiles were adjusted to different mathematical models to find the best fit. Higuchi diffusion and Peppas power law models (eq. 6 and 7) were used, they were user defined (39).

$$\frac{Q_t}{Q_\infty} = k \cdot \sqrt{t} \qquad \text{(Eq. 6)}$$

$$\frac{Q_t}{Q_\infty} = k \cdot t^n \qquad \text{(Eq. 7)}$$

Q_t – released amount at time t

Q_∞ – released amount at the end of study

k – constant

t – time in h

n – constant

Statistical analysis was done with SPSS 14.0 for Windows Evaluation version. One way and two way analysis of variance were used to compare loading capacity and release kinetics of different lenses in different media after verifying normality and homogeneity of variance.

4 Results and discussion

4.1 Lens formulation

The two most used materials for fabrication of soft contact lenses are pHEMA and silicone based hydrogels. For our work we selected soft contact lenses based on pHEMA hydrogels cross-linked with EDMA, because these had already been used as a support for the release of different drugs. Comonomers MA and NVP were included in their composition because they are known to increase the water uptake properties of pHEMA hydrogels, improving their mechanical properties, which allow the lenses to resist the force of the eye lid and also elevate the oxygen permeability (23). All comonomers used are soluble in HEMA at all proportions and also soluble in water therefore no changes in monomer solution's aspect were appreciated at any time.

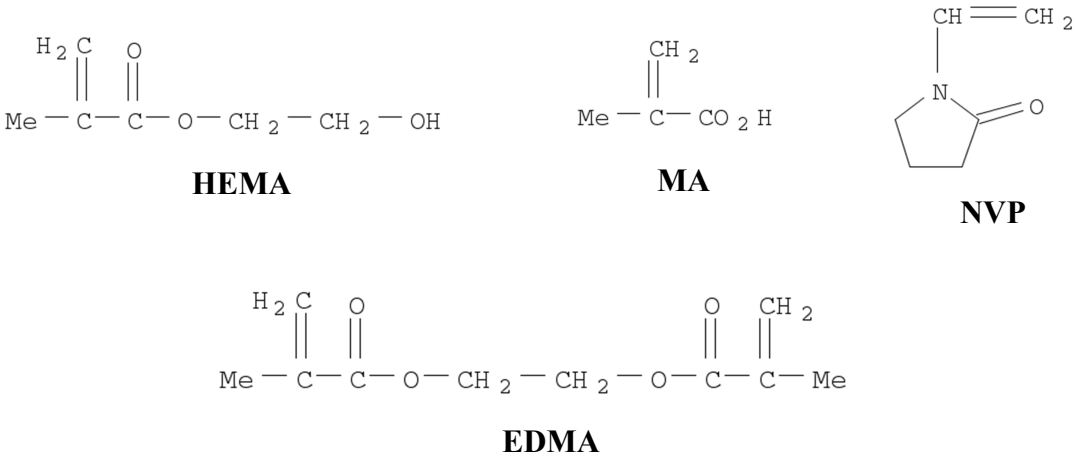


Figure 11: Structure of the HEMA, crosslinker EDMA and comonomers MA, NVP (35).

After the thermal polymerization all hydrogels were rigid and fragile while porous hydrogel sheets obtained by UV polymerization were more flexible and flexibility augmented with water content. The reason lies in the different conditions used in the thermally induced polymerization in comparison with photochemically induced process, which allow important differences in water content of hydrogels at the end of polymerization. When hydrogels are prepared by radical solution polymerization with thermal initiation, no water is added to the monomer mixture and the use of high temperature (50 and 70 ºC for 12 and 24 h) produces more cured systems. When they are prepared by UV polymerization however the water content hardly changes therefore the resulting hydrogels are partly hydrated hence more flexible. Nevertheless, once the lenses

prepared by thermal-polymerization were fully hydrated they became perfectly flexible and soft (figure 12).

Figure 12: Fully hydrated hydrogel sheet on the left and hydrogel sheet residue after lens cutting on the right.

At the end of the polymerization the hydrogels sheets were boiled, cut into small discs (Φ = 10 mm) and left in washing solutions for several days. The cleaning procedure was over when there were no monomer residuals left in washing solution, which could be seen as the absence of absorption peaks in the range 190 – 300 nm where the characteristic UV absorption bands are (figure 13).

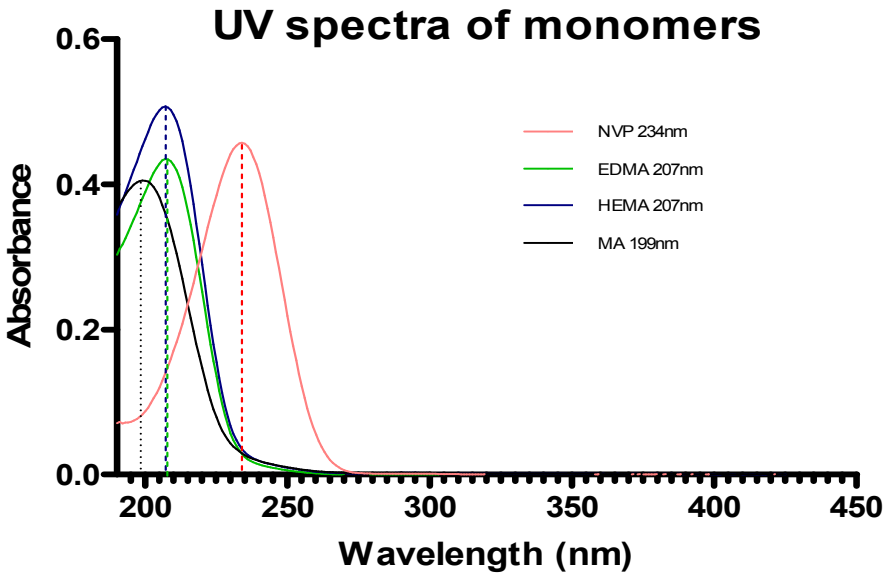


Figure 13: Characteristic absorption bands of the monomers used for preparation of hydrogels - recorded in water solutions.

Resulting lenses had a clear transparent appearance in dry and hydrated state. The transmittance at 600 nm was recorded with fully hydrated and drug loaded samples. Light transmission was over 90% in all cases, which is the value that the manufacturers usually obtain.

4.2 Non-porous lenses

4.2.1 Lens characterization

Hydrogel surface was observed by scanning electron microscope at amplifications 500x and 1000x. SEM micrographs of hydrogels prepared by thermal polymerization showed a homogeneous surface (figure 14).

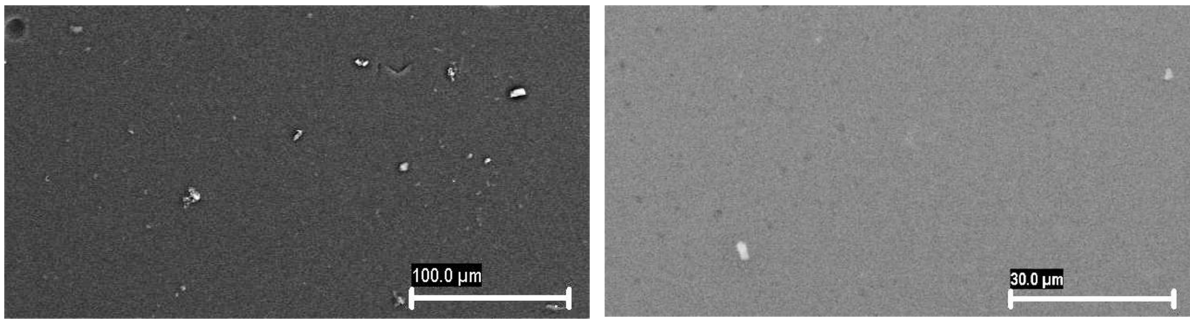


Figure 14: SEM micrographs of hydrogels prepared by thermal polymerization. NVP200 hydrogel surface (left, 500x amplification) and MA200 hydrogel surface (right, 1000x amplification)

4.2.1.1 Swelling kinetics

Next step in lens characterization was to study the water uptake and swelling behavior of the hydrogels. With this aim we prepared the swelling profiles in physiological saline solution and PBS buffer adjusted to pH 7.4, which were also used in drug loading experiments. These media were chosen because they are normally used as lens packaging solutions. At first only the behavior of non-porous lenses prepared by thermal polymerization was analyzed.

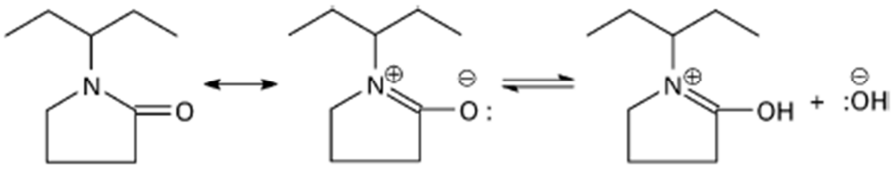
Table V: Average swelling percentage and equilibrium water contents of completely swollen lenses immersed in 0,9% NaCl solution or PBS buffer (n = 6).

Lens	%EWC (mean ± SD) 0,9% NaCl	%EWC (mean ± SD) PBS buffer
0	47,5 ± 1,1	47,3 ± 4,7
MA100	49,0 ± 2,8	58,8 ± 4,2
MA200	48,0 ± 1,6	65,6 ± 1,9
NVP100	52,4 ± 1,4	57,4 ± 1,5
NVP200	51,4 ± 0,7	55,2 ± 2,2

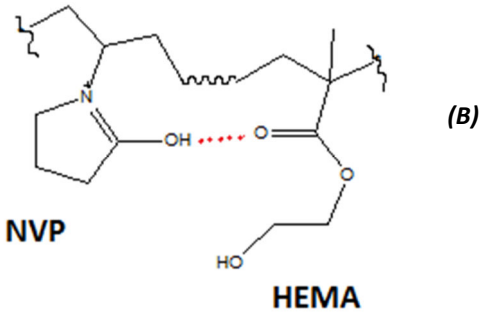
The profiles were analyzed with analysis option of the GraphPad 5.0 software. All the lenses were completely swollen in 24 h or less whatever the medium used. In the saline physiological solution the EWC were very similar (table V). However the analysis of variances (ANOVA) showed that the differences between groups were significant ($\alpha<0,05$). NVP hydrogels have the highest degree of swelling, followed by the MA hydrogels. NVP and MA are both known to be hydrophilic monomers that improve swelling properties of lenses. Hydrogels 0, which contain no comonomer have the lowest degree of swelling.

It had been described before that the water uptake by hydrogels with ionized moieties, in this example MA100 and MA200 (methacrylic acid), depends on the pH and ionic strength of the medium, content of ionizable groups and cross-linking of the hydrogel. In our particular case the cross-linking in all hydrogels was kept constant, the only variable were the functional monomers used. In PBS buffer 7,4 carboxyl groups of MA residues (pK_a 4,66) ionize (COO^-) generating repulsive forces within the hydrogel. At this pH more than 99% of MA is ionized and that is why the MA100 and MA200 equilibrium water content is significantly higher in PBS than it is in NaCl ($\alpha<0,05$). The EWC increased with the proportion of MA and MA200 lenses showed a particularly high degree of swelling, which

could be seen in their dimensions and their EWC values (figures 16, 17). In the case of NVP hydrogels the swelling seems to be affected by the change of medium as well but the increase was not that marked. A well-documented property of NVP in the presence of hydrogen donors such as water is a shift of the tautomerism as shown in figure 15 (40). Formation of hydrogen bonds between the hydroxyl groups of NVP residues and carbonyl groups of HEMA is therefore possible in aqueous solutions such as 0,9% NaCl and PBS buffer (figure 15). These bonds would in some degree restring hydrogel expansion. The PBS buffer's higher pH and ionic strength values might result in fewer hydrogen bonds; therefore the hydrogel could swell more. Swelling kinetics was unchanged for the hydrogels 0, with no functional monomer.



(A)



(B)

Figure 15: The lactam – lactim tautomerism of NVP could lead to hydrogen bond formation within the hydrogels fabricated with NVP. (A) Lactam - lactim tautomerism of NVP as reported in earlier works (40). (B) Scheme representing the possible hydrogen bond formation between NVP and HEMA residues in our hydrogels when these are submersed in aqueous solutions.

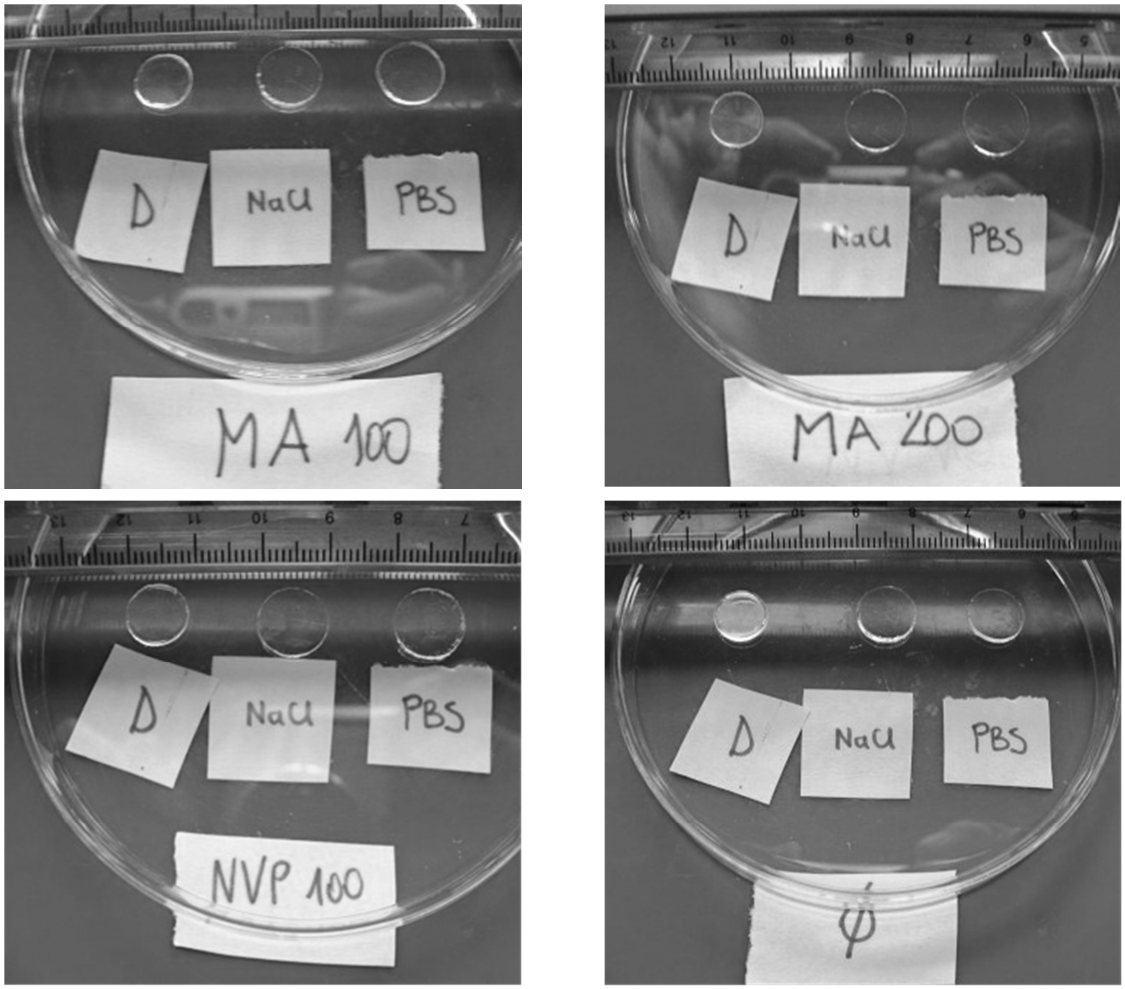


Figure 16: Photos of lenses in dry and swollen state after swelling in 0,9% NaCl or PBS buffer.

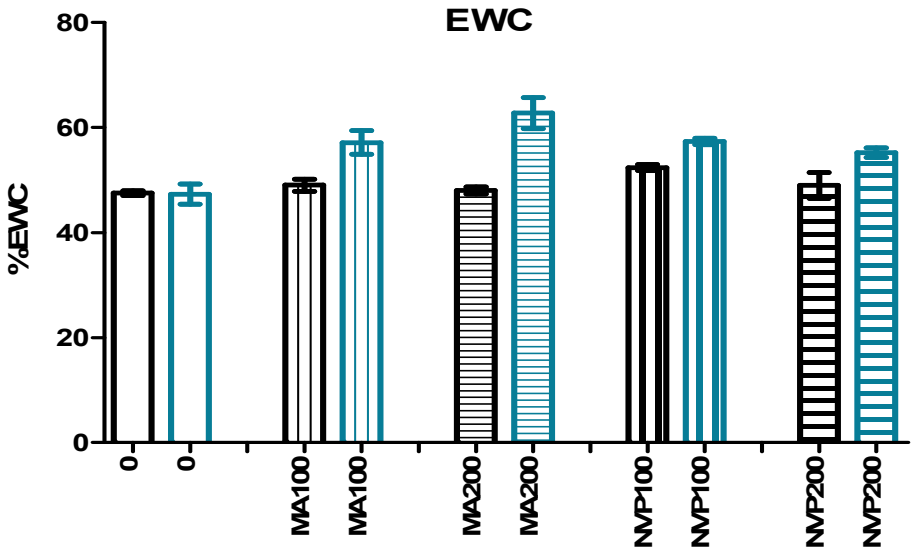


Figure 17: Graph representing the %EWC in both solutions. Black columns correspond to 0,9% NaCl and blue to PBS buffer.

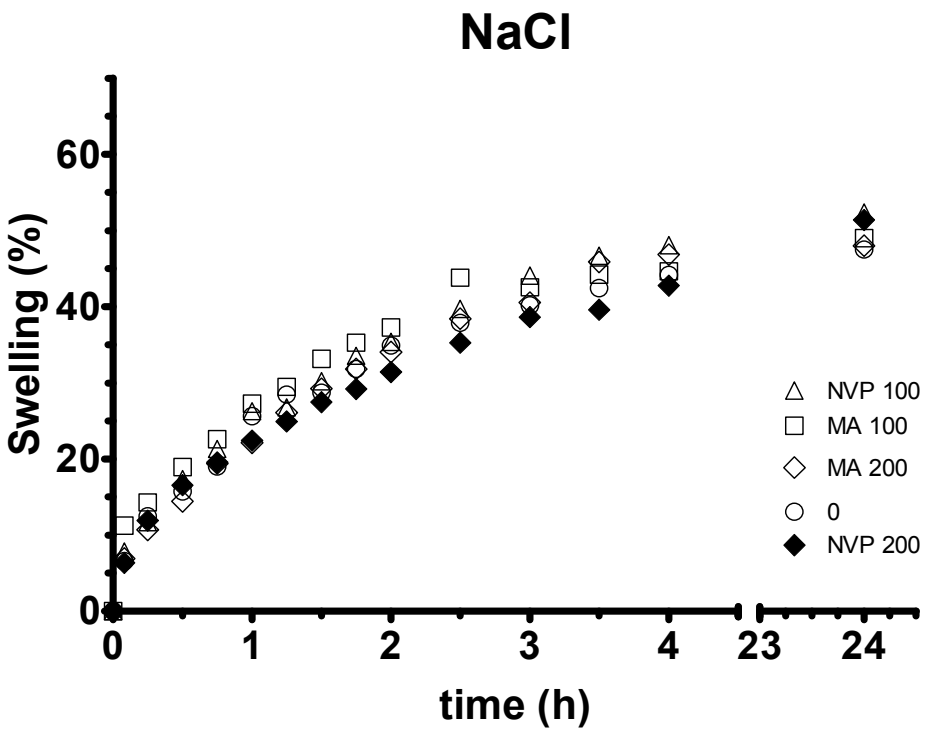


Figure 18: Swelling profiles of lenses in physiological saline solution.

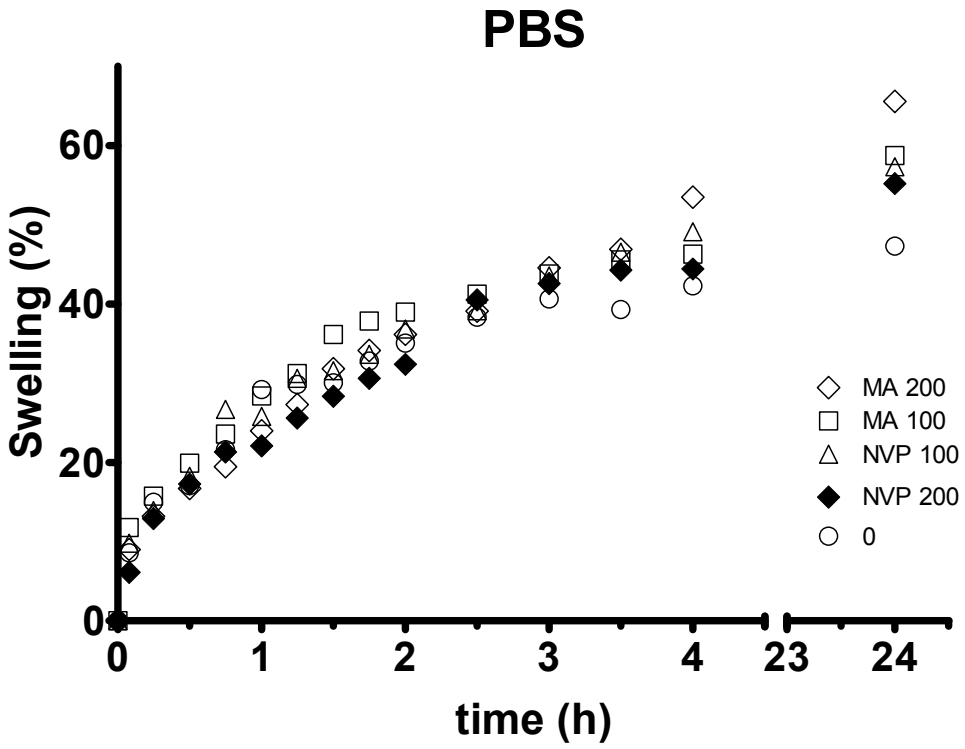


Figure 19: Swelling profiles of lenses in PBS buffer (pH 7,4).

All the profiles (figures 18 and 19) in the range of 0 – 75% EWC depend linearly on $\sqrt{t}$, which means that Fickian behavior was maintained in spite of the swelling of polymeric network or group ionization within hydrogels as it had already been reported in earlier works (30).

4.2.2 Drug loading and in vitro drug release

4.2.2.1 Calibration curves

UV-VIS spectrophotometrical analytical methods were used to analyze our samples. Linearity is one of the essential parameters for the validation of analytical method since it allows us to derive a mathematical equation describing a relationship between the parameter (concentration) and the response (absorbance). Several calibration curves were prepared from different bulk drug solutions. Linearity of the method was evaluated by linear regression using the least squares approach. Linear regression function of the GraphPad program was used. Rather than forcing the line through the origin, a 95% confidence interval was observed to assure the origin is included, which is an indicative of the absence of analytical bias. Equations with square of Pearson coefficient and α value for the slope, as obtained by ANOVA of the model, are gathered in table VI. Regression lines with 95% of the confidence interval (CI) are represented in figure 20.

In drug release studies sink conditions had to be maintained during the experiment therefore in lachrymal fluid mainly low concentrations were analyzed to get a more sensible calibration curve.

Table VI: Equations of calibration curves of TA in different solutions.

Media	Model equation	95% CI	
		y intercept (x = 0)	x intercept (y = 0)
0,9% NaCl	$c\left(\frac{\mu g}{ml}\right) = \frac{A - 0{,}0020}{0{,}0346}$ $R^2 = 0{,}997$ $\alpha < 0{,}0001$	-0.005617 to 0.009713	-0.3635 to 0.2381
PBS (pH 7,4)	$c\left(\frac{\mu g}{ml}\right) = \frac{A + 0{,}0042}{0{,}0363}$ $R^2 = 0{,}997$ $\alpha < 0{,}0001$	-0.0157 to 0.00735	-0.286 to 0.502
LF (pH 8)	$c\left(\frac{\mu g}{ml}\right) = \frac{A - 0{,}0016}{0{,}0361}$ $R^2 = 0{,}998$ $\alpha < 0{,}0001$	-0.0004180 to 0.003624	-0.1394 to 0.04956

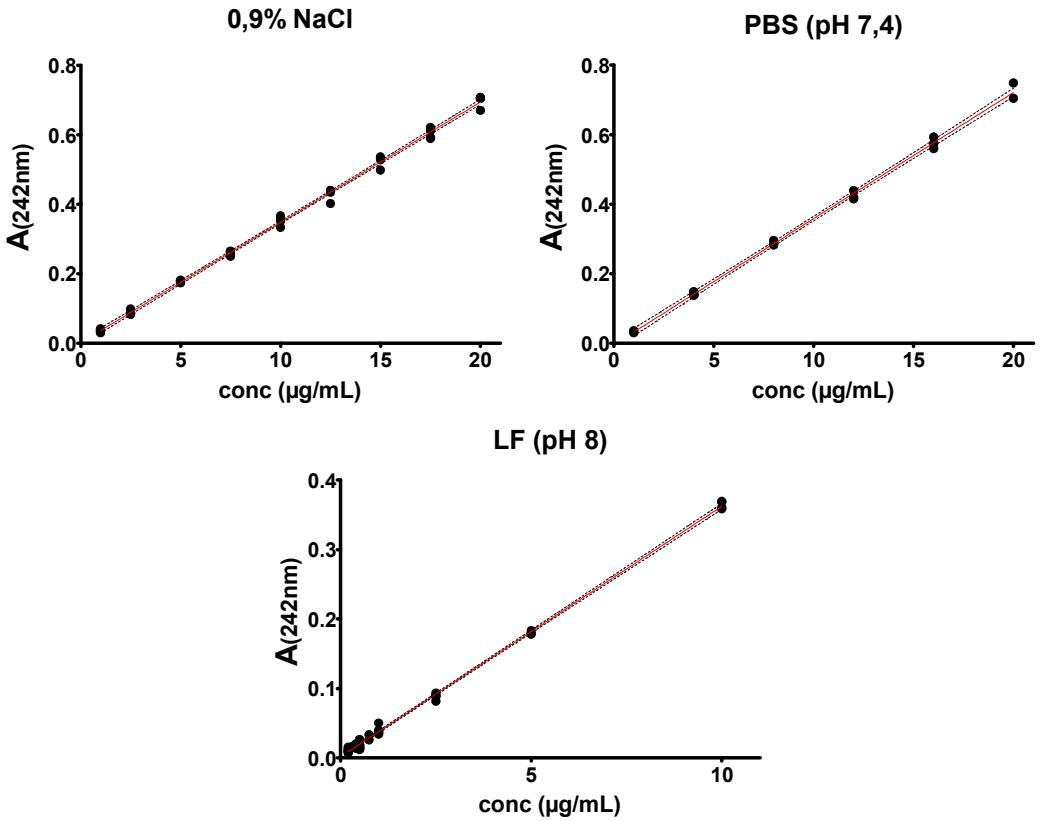


Figure 20: Calibration curves with 95% CI for TA dissolved in different media. LF - lachrymal fluid.

4.2.2.2 Loading studies

The first loading experiments were carried out in physiological saline solutions of drug. Different concentrations were assayed to obtain the information about the adsorption isotherm. Concentration range was from about 8 to 19 µg/ml, which was the maximum solubility of TA in saline medium.

Others have described that binding of the drug to the hydrogel can be modeled as a Langmuir adsorption isotherm, which relates the adsorbed concentration of the drug on the gel to the free concentration in the aqueous phase inside the gel by the following equation:

$$C_{gel} = \frac{C_{gel}^{\infty} \cdot C_{aq}}{\mathrm{k} + C_{aq}} \qquad \text{(Eq. 8)}$$

where C_{gel}^{∞} is the surface concentration in the hydrogel at the maximum packing and k is the ratio of the rate constants for desorption and adsorption of the drug on the HEMA surface hydrogels (38).

Hydrogels behave as microporous adsorbents in the drug loading process, which normally produces adsorption isotherms of class C according to the classification proposed by Giles et al (41). These isotherms are distinguished by an initial linear portion which implies a constant partition of the solute between the solute and the adsorbent. These kinds of class C isotherms are related with adsorbates that penetrate within the adsorbent, hence disappear from the surface. In this case the critical concentration C_{gel}^{∞} represents the saturation of sites in internal area of the adsorbent and is characterized by a plateau in the adsorption isotherm.

As can be seen from figure 21 our loading isotherms mostly adjusted well to a straight line, meaning that we are still in the initial zone of the Langmuir adsorption isotherm, therefore far away from the saturation of adsorption sites. The slopes of the straight line correspond to the partition coefficient, the values of which are shown in table VII.

The drug loading was expressed as the amount of drug loaded per weight of dry lens (mg/g). It was the lowest in the 0 hydrogels, significantly lower than with MA lenses (α<0,05). There were no significant differences between the NVP and MA lenses therefore we assumed that there were no special interactions between the drug and the comonomers. Since it was in both cases higher than for the 0 lenses we assumed that it could be related to higher water content.

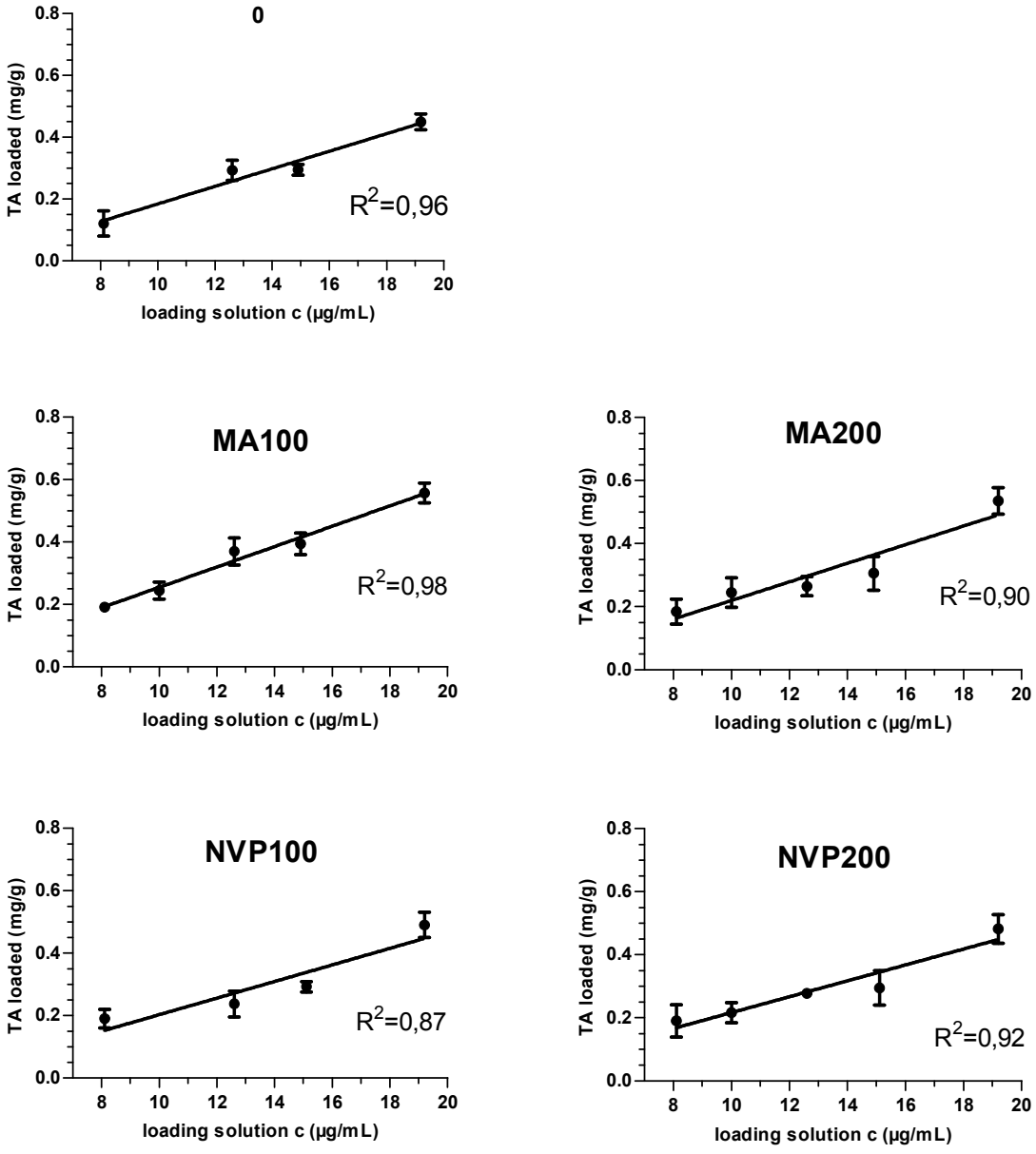


Figure 21: Adsorption isotherms for NaCl drug loading solutions.

The tendency of the drug for the polymeric network was calculated by the partition coefficient, which is defined as the ratio of the drug concentration in the gel and the concentration in the aqueous phase at equilibrium. The results (table VII) imply that in all hydrogels a large fraction of drug is bound to the polymeric network. Similar results have been obtained for another corticosteroid drug, the dexamethasone (38). From their values it can be concluded that the drug has a similar affinity for the three hydrogel networks.

Table VII: Maximum loaded amount of TA in lens and partition coefficients (n = 6) obtained in drug loading studies with physiological and buffered drug solutions.

Loading solution	Lens	Amount loaded (mg/g) mean ± SD	K_p mean ± SD
0,9% NaCl	0	0,449 ± 0,026	22,9 ± 0,8
	MA100	0,557 ± 0,031	27,3 ± 2,0
	MA200	0,536 ± 0,042	26,0 ± 3,4
	NVP100	0,491 ± 0,040	23,4 ± 2,5
	NVP200	0,506 ± 0,072	24,5 ± 3,0
PBS pH 7,4	0	0,465 ± 0,020	23,8 ± 2,5
	MA100	0,585 ± 0,044	24,9 ± 3,1
	MA200	0,667 ± 0,038	27,2 ± 1,7
	NVP100	0,501 ± 0,068	25,2 ± 4,0
	NVP200	0,583 ± 0,035	27,8 ± 3,5

- ***Effect of pH value on loading capacity of lens***

Subsequent experiments were performed with PBS drug solutions with pH adjusted to 7,4. It had already been seen that the swelling is affected by the pH especially in the case of MA lenses which incorporate comonomer with ionizable groups. The results of the previous study (drug loading in physiological drug solution) suggested the loading was mostly related to the EWC of the hydrogels so the following experiments were conducted in order to confirm these assumptions. The procedure was identical. To be able to do a statistical comparison, the experiments were conducted at equal conditions, with loading solutions of almost equal concentration range.

Again the isotherms adjusted well to a straight line, which was expected since the drug was used within the same concentration range. Pearson coefficients obtained were better than before. The slopes were steeper signifying that the amount loaded increases with the

concentration of loading solution much faster. Drug loading was significantly augmented in the case of MA200 which corresponds with the results obtained in swelling studies. It was interesting to note that the amount loaded was also significantly improved in the case of NVP200 lens but it did not come to reach the levels of MA200. The effects of pH change on loading can be observed in figure 22. Partition coefficients were calculated as well, to see if the affinity for the network changes in any way but the results were similar to the previous ones (table VII).

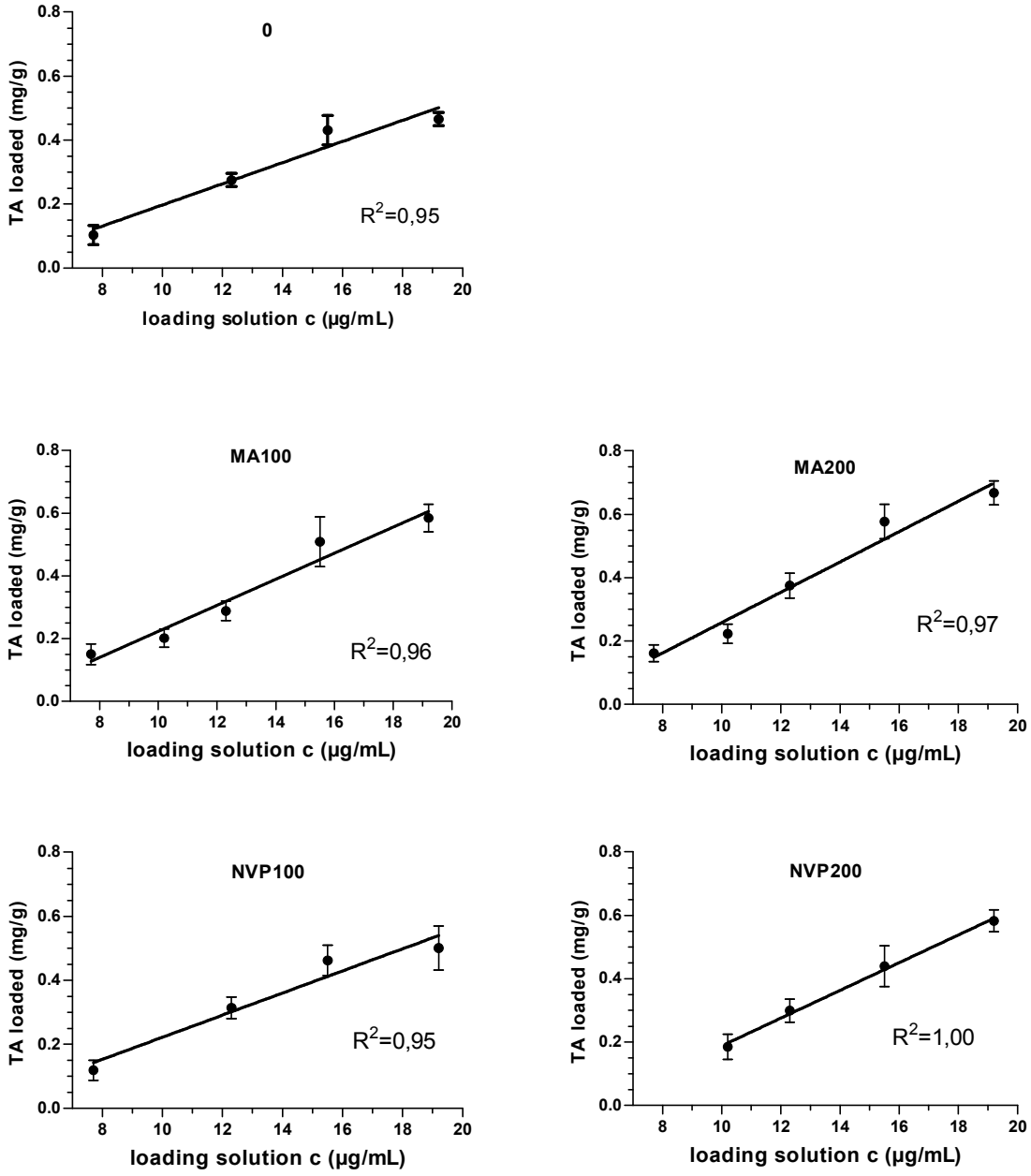


Figure 22: Adsorption isotherms for PBS drug loading solutions.

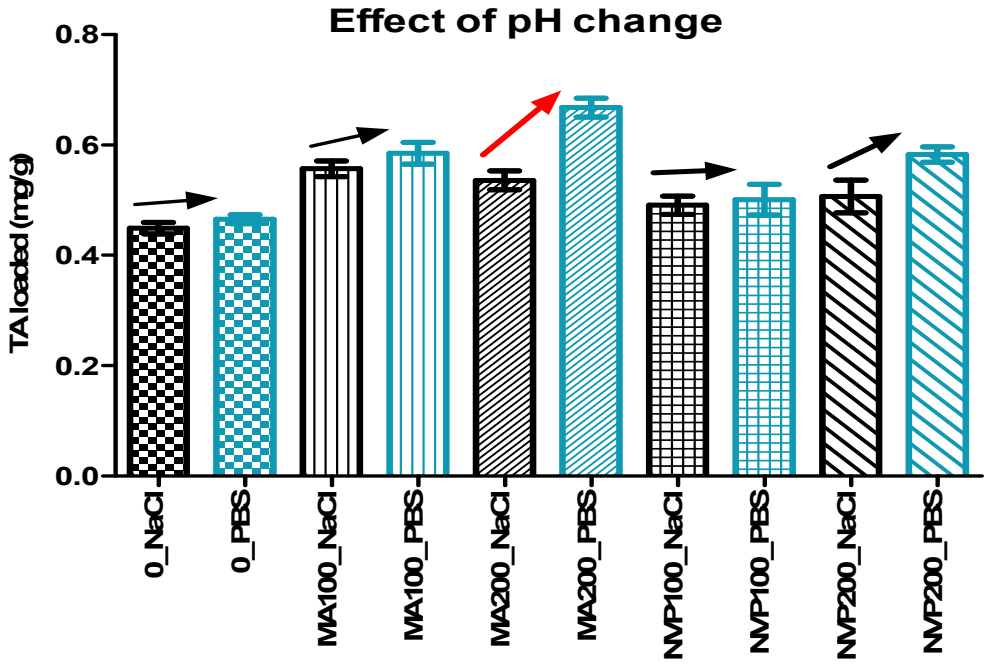


Figure 23: Drug loading improvement following the change of drug solution (c = 19μg/ml). Arrows represent the tendency of drug loading improvement upon change of the drug loading solution. Drug loading was significantly improved in the case of MA200 lenses (red arrow).

4.2.2.3 Drug release

Release profiles of the drug loaded lenses are shown in figure 24. All samples began to release the drug as soon as they were submersed and no drug retention or lag time was observed. Once again we could conclude that there is no special affinity of the drug for any of the monomers. All hydrogels show similar release profiles with the same release rate during the first few hours but significant differences were found after 5 hours of the experiment. Results show that eventhough there are no differences in the affinity between hydrogels and the drug, those made with MA produced higher release rates. MA100 and MA200 have released the drug faster than the other three in accordance with their swelling profiles. At this pH more than 99% of the functional groups of MA are ionized generating repulsive forces within the hydrogel. This results in higher swelling and network relaxation which facilitates TA release. All samples released 100% of the drug loaded. In the case of MA lens all the drug was released in about 48 h, while other lenses released it in about 72 h.

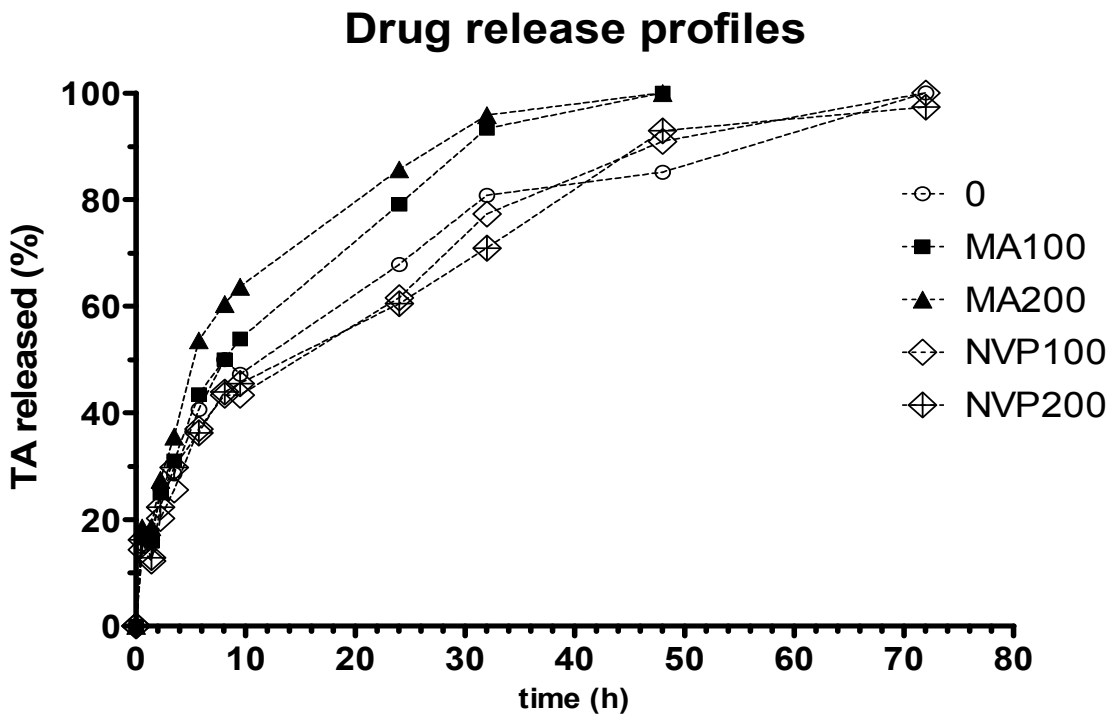


Figure 24: Drug release profiles obtained for lenses prepared by thermally induced polymerization.

To analyze the main mechanism involved in the drug release process, release profiles were analyzed individualy. Drug release profiles were adjusted to two release models: Kosmeyer and Peppas equation and Higuchi model. Peppas power law was used in the early 60% of drug released, to obtain the exponent n characterizing the drug release mechanism. Three different transport cases are possible depending on the mechanism controlling the release (42).

Table VIII: Values of n exponent and relations to the drug transport mechanism from slabs geometry (39, 42).

Exponent value	Case transport	Release mechanism
n = 0,5	Case I	Pure diffusion controlled release
n = 1	Case II	Swelling controlled release
0,5 < n < 1	Anomalous kinetics	Combination of swelling and diffusion controlled release

The values of all exponents were about 0,5 with a small degree of deviation. We therefore assumed that we have a case I transport mechanism, which made sense considering we were dealing with fully hydrated samples. In this case desorption of the drug molecules from the hydrogel and their diffusion into the bulk solution are the main mechanisms responsible for the release process. Degree of fit to Higuchi equation in the first 75% release was also analyzed. Results are gathered in table IX. All profiles adjusted well to this model ($R^2 > 0,97$) meaning that diffusion really is the driving force of drug release. Analysis of variances was performed for the drug release rates (K). Results show significant differences ($\alpha<0,05$) between samples (Kruskal-Wallis nonparametric test) Post-hoc tests (Tamhane) show that significant differences could be found between MA200 with the highest K and 0, NVP100 and NVP200 lenses, but that there were no significant differences with MA100 lens.

Table IX: Results obtained after adjusting the release profiles to Higuchi equation (n = 12).

Lens	Square of Pearson coefficient (R^2)	Higuchi constant K [h^{-1}] Mean ± SD
0	0,97	0,138 ± 0,006
MA100	0,98	0,171 ± 0,018
MA200	0,98	0,193 ± 0,020
NVP100	0,97	0,132 ± 0,007
NVP200	0,97	0,127 ± 0,009

In the following figure (fig. 25) the case of drug release after drug loading in PBS solution with TA concentration 19 µg/ml is represented. It could be seen that the lenses MA100 and MA200 can release noteworthy amounts comparing to the other lenses. In the case of MA200 the released amount was almost twice as much as with NVP and 0 lenses.

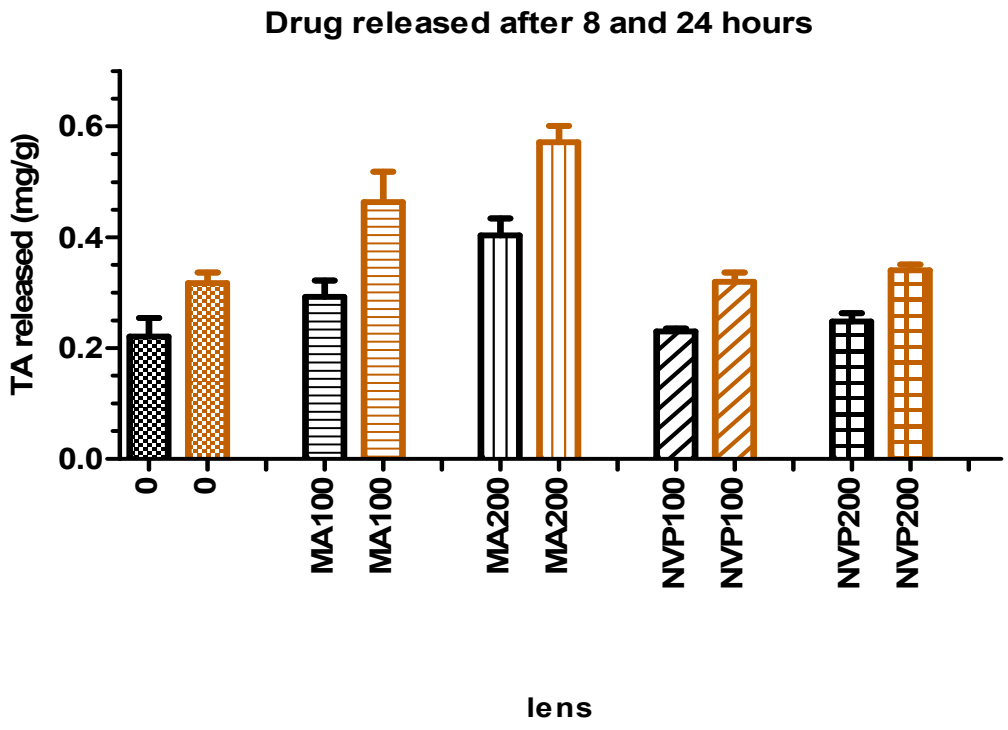


Figure 25: Amount of drug released (mg of drug per g of lens) in the first 8 (black columns) and 24 h (brown columns).

4.2.2.4 Reproducibility of drug release kinetics

One of the advantages that lens are supposed to present is reproducibility of drug release kinetics hence the patient could use the same lenses during various days of therapy, after charging the lenses by means of repetitive drug loading. In order to verify that our lenses exhibit reproducible release kinetics after multiple drug loadings, we did a second drug liberation experiment after submersing the lenses for four days in the same drug solution as used in the first loading process.

Drug release curves show that TA was completely released in the same time periods determined in the first release. The drug levels and release kinetics in the lachrymal fluid were similar. Release rate constants obtained after fitting the data to Higuchi diffusional model did not differ significantly from the results of the first release experiment.

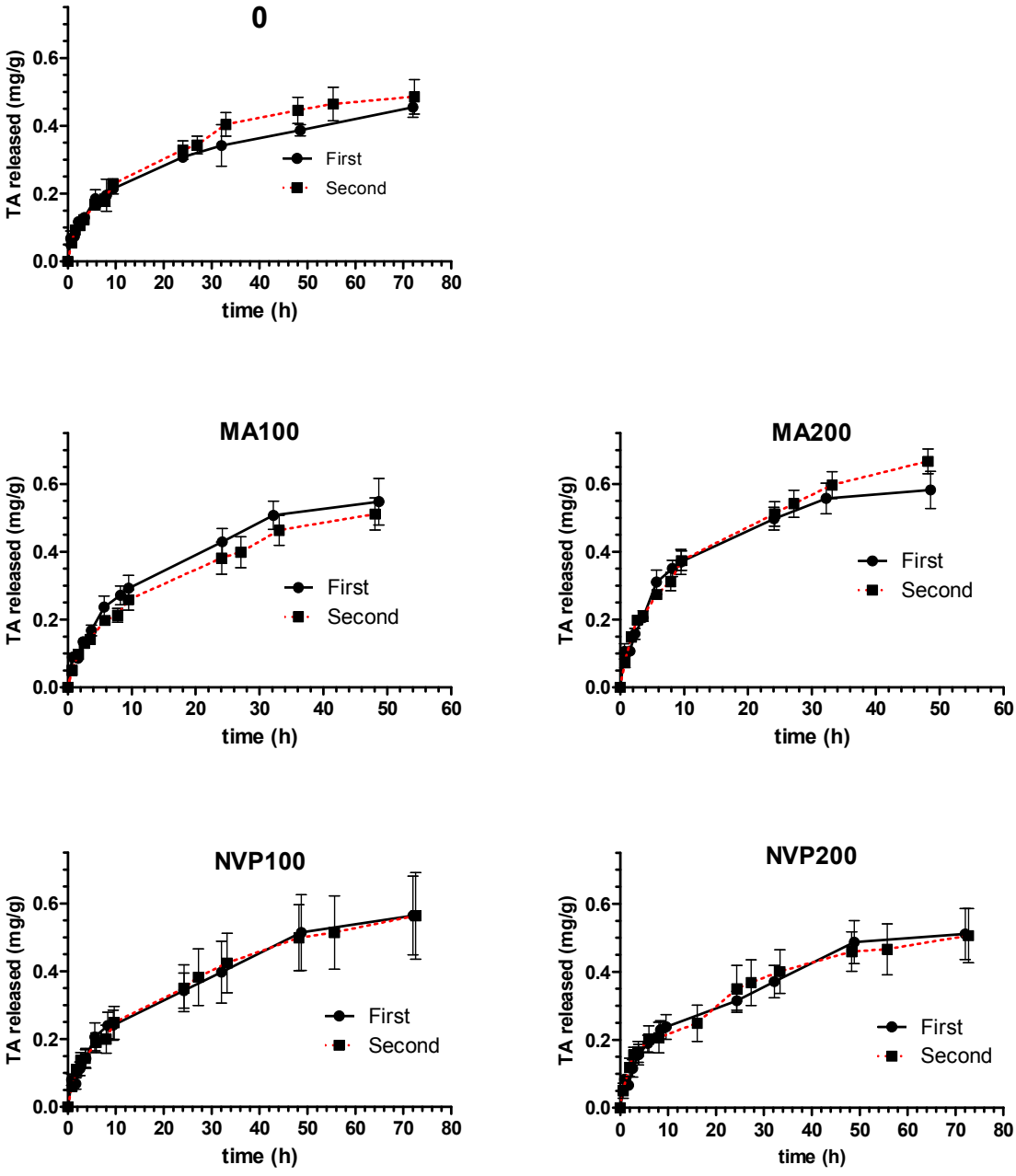


Figure 26: Drug release profiles from lenses after first and second loading process. It can be appreciated that these overlap, meaning the release kinetics is reproducible.

4.3 Porous lenses

Porous lenses were prepared by photo-polymerization of mixtures of monomers incorporating water and their microstructure, drug loading capacity and drug release behavior were analyzed.

4.3.1 Lens characterization

In their macroscopic aspect no differences between the non-porous and the porous hydrogels could be appreciated, hence observation with a scanning electronic microscope (SEM) was done. SEM images (fig. 27) of the lens surface revealed that hydrogels prepared by photochemical polymerization in the absence of water had a homogeneous surface with hardly any pores covering it similar to that obtained from thermo-polymerization. On the other hand as expected, hydrogels prepared with 20 vol% and 40 vol% of water both presented pores on their surfaces, the proportion and size of which depended on the water content in the monomer solution.

Size distribution of the superficial pores, which had presented on the surface of lenses prepared in the presence of water, is shown in figure 28 and table X. All the lenses exhibited unimodal normal pore size distribution with the exception of the ma200_p20 where a clear bimodal distribution with mean Feret diameter of 1,442 ± 0,026 and 4,407 ± 0,1748 μm was obtained.

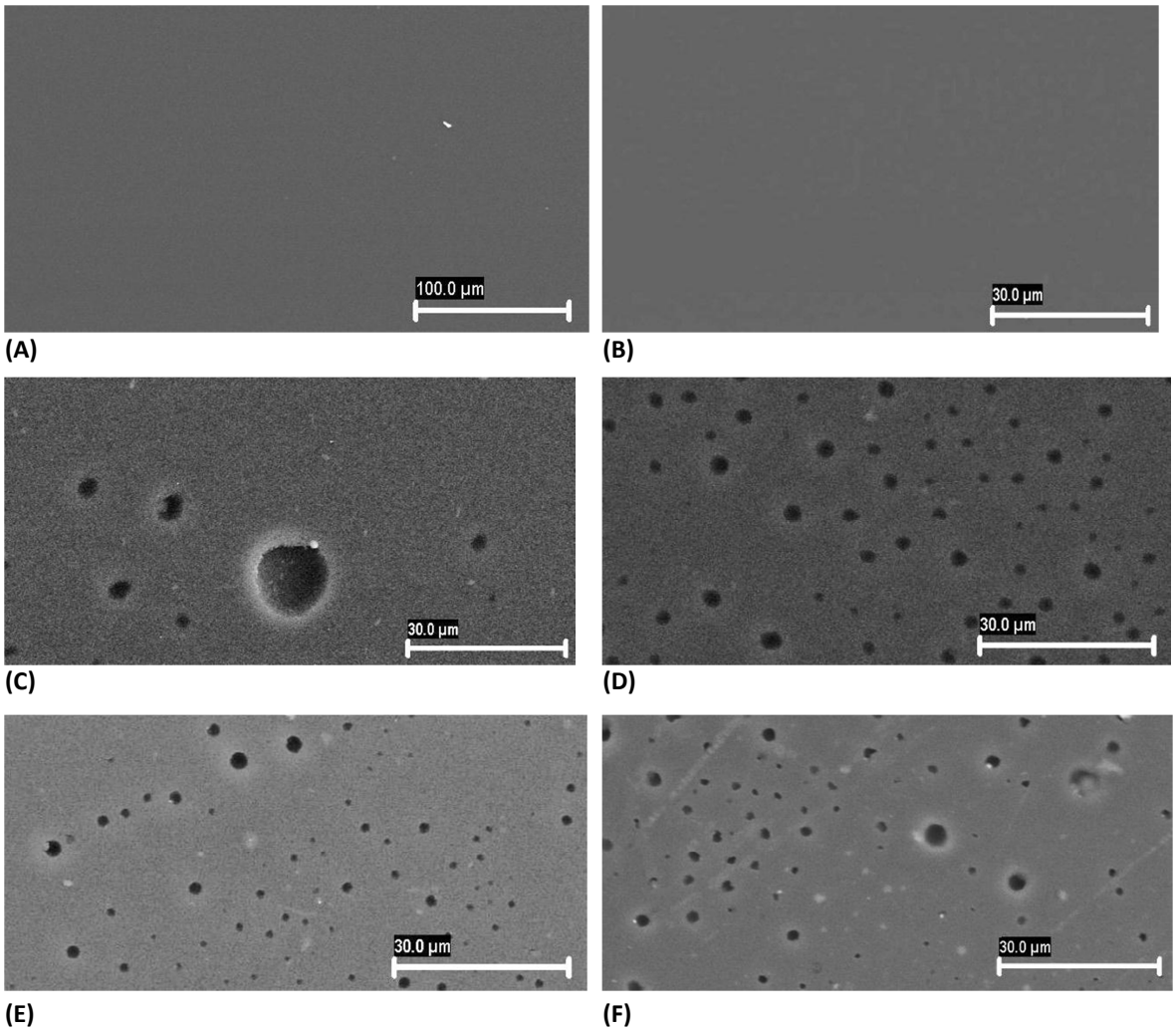


Figure 27: SEM micrographs revealing the surface of lenses prepared by photochemical polymerization without and with different proportions of water. Surface of non-porous ma200_p0 hydrogels, 500x (A) and 1000x amplification (B). The following images were taken at 1000x amplification and are as follows: ma200_p20 hydrogel surface with bimodal pore distribution (C), ma200_p40 (D), nvp200_p20 (E) and nvp200_p40 (F) hydrogel surfaces.

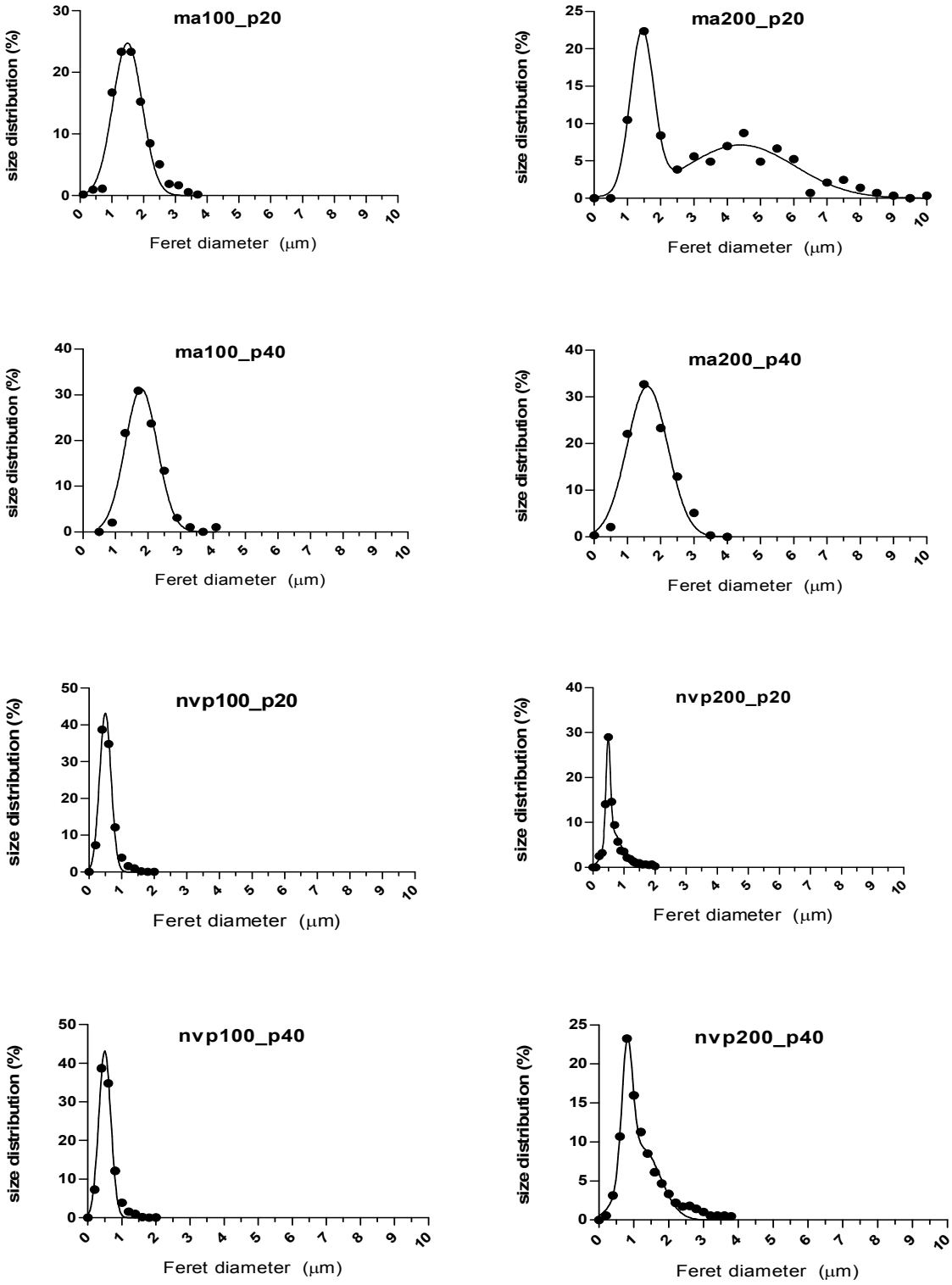


Figure 28: Graphs of surface pore size distribution estimated from SEM images for porous lenses.

Table X: Surface pore mean size for lenses formulated by photo-polymerization with incorporation of water in the monomer solution.

Lens	Feret diameter (μm) mean ± SD	95% CI of the mean	% area occupied by pores at the surface of the hydrogels
ma100_p20	1,66 ± 0,75	1,60 to 1,73	2,64 ± 0,28
ma100_p40	2,07 ± 1,22	1,82 to 2,32	1,68 ± 1,34
ma200_p20	4,29 ± 9,38	3,20 to 5,38	3,74 ± 3,82
ma200_p40	1,74 ± 0,69	1,68 to 1,79	3,09 ± 1,50
nvp100_p20	0,58 ± 0,37	0,56 to 0,60	2,05 ± 1,46
nvp100_p40	0,81 ± 0,61	0,75 to 0,88	1,61 ± 0,49
nvp200_p20	0,78 ± 1,16	0,72 to 0,85	1,74 ± 0,61
nvp200_p40	1,35 ± 1,37	1,27 to 1,44	1,67 ± 0,65

In general the use of water in lenses elaborated with MA produces larger surface pores than in those elaborated with NVP. Also the increase of water content in the monomer mixture leads to a more porous surface with larger pores.

After a statistical evaluation of results obtained in previous experiments (non-porous lenses) the best possible combination of hydrogel composition and media was selected to evaluate the effect of porosity on drug loading and release. This was determined to be MA200 in PBS drug solution. This time the PBS buffer was adjusted to 8 in order to avoid fluctuations between drug load and drug release studies. Augmenting pH by 6 decimals didn't affect our experiment because carboxyl groups had already been ionized in more than 99% at pH 7,4 already and the drug is stable at this pH. The three porosity types were evaluated (ma200_p0, ma200_p20, ma200_p40).

4.3.1.1 Swelling

Increase in swelling as the porosity augments had been reported in previous works. They concluded that the size of pores and the surface they cover have an important impact on swelling properties of hydrogels (37, 43, 44). Lenses were submersed in PBS pH 8 buffer, since the loading studies were going to be carried out in this solution as well. All the lenses were completely swollen in 24 h. With the proportion of water in monomer solution the

percentage of surface covered with pores augmented as it was determined by SEM image analysis. In agreement with the increase in porosity the EWC increased as well. Samples ma200_p40 had more than 20% higher EWC than the ma200_p0 samples. We noted that the EWC of ma200_p20 and nonporous ma200_p0 didn't reach the EWC of the MA200 prepared with thermal polymerization (table XI) probably because of different polymerization conditions (temperature, process duration). The profiles again adjusted very well to Higuchi model ($R^2 > 0,98$).

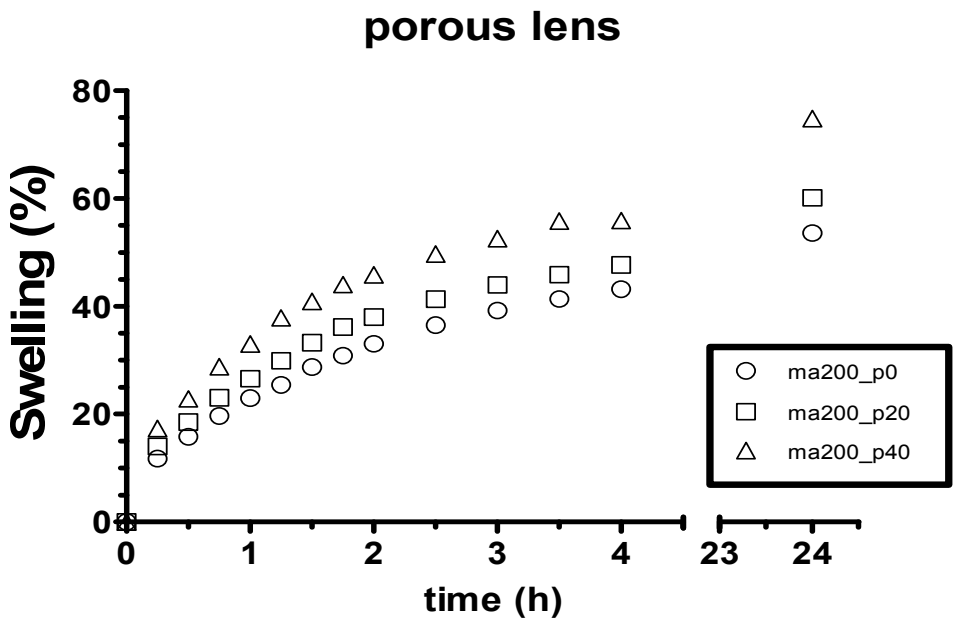


Figure 29: Swelling profiles in PBS buffer pH 8 for porous lenses prepared by UV-polymerization.

Table XI: Equilibrium water contents after swelling in PBS buffer (pH 8) solution (n = 6).

Lens	Polymerization type	%EWC (PBS buffer) Mean ± SD
ma200_p0	UV polymerization	53,6 ± 1,0
ma200_p20		60,1 ± 1,0
ma200_p0		74,8 ± 1,7
MA200	thermal polymerization	65,6 ± 1,9

4.3.2 Effect of microstructure on loading capacity and drug release kinetics

Lenses were submersed in 10 ml buffered drug solution with concentration 19 µg/ml. After four days the concentrations of the surrounding solutions and lens dimensions were determined. Lenses ma200_p40 were a little bigger in diameter but the thickness was practically equal. The amount of drug loaded augmented in agreement with porosity (table XII). One way ANOVA with post hoc test for unequal variances was performed on the results. Analysis showed that samples ma200_40 loaded a significantly larger amount of TA than ma200_p0. Between the ma200_p20 and ma200_p0 the difference was not significant. Therefore we can conclude that the percentage of surface covered with pores impacts greatly the loading capacity of our hydrogels and that the capacity significantly augments as the water content in the monomer mixture reaches 40 vol%.

Table XII: Maximum amount of drug loaded in lens prepared with UV-polymerization and partition coefficients (n = 6).

Lens	Amount loaded (mg/g) Mean ± SD	K_p Mean ± SD
ma200_p0	0,635 ± 0,075	26,7 ± 4,0
ma200_p20	0,731 ± 0,045	28,3 ± 2,8
ma200_p40	0,789 ± 0,072	28,3 ± 2,9

4.3.2.1 In vitro drug release study

The release process was finished in 48 h for all samples, there was no retention period. The profiles (fig. 30) in the range 0 – 75% of release all adjusted well to Higuchi model ($R^2 \geq$ 0,97) therefore the rate constants could be used for group comparison. ANOVA analysis showed the rate of drug release was significantly higher with porous samples (ma200_p20 and ma200_p40). This rate however did not differ from that obtained in case of MA200 lens fabricated by thermal curing.

Table XIII: Result obtained after adjusting the release profiles to Higuchi equation (n = 6)..

Lens	Square of Pearson coefficient (R^2) Mean	Higuchi constant K [h^{-1}] Mean ± SD
ma200_p0	0,99	0,167 ± 0,009
ma200_p20	0,98	0,186 ± 0,005
ma200_p40	0,99	0,182 ± 0,005

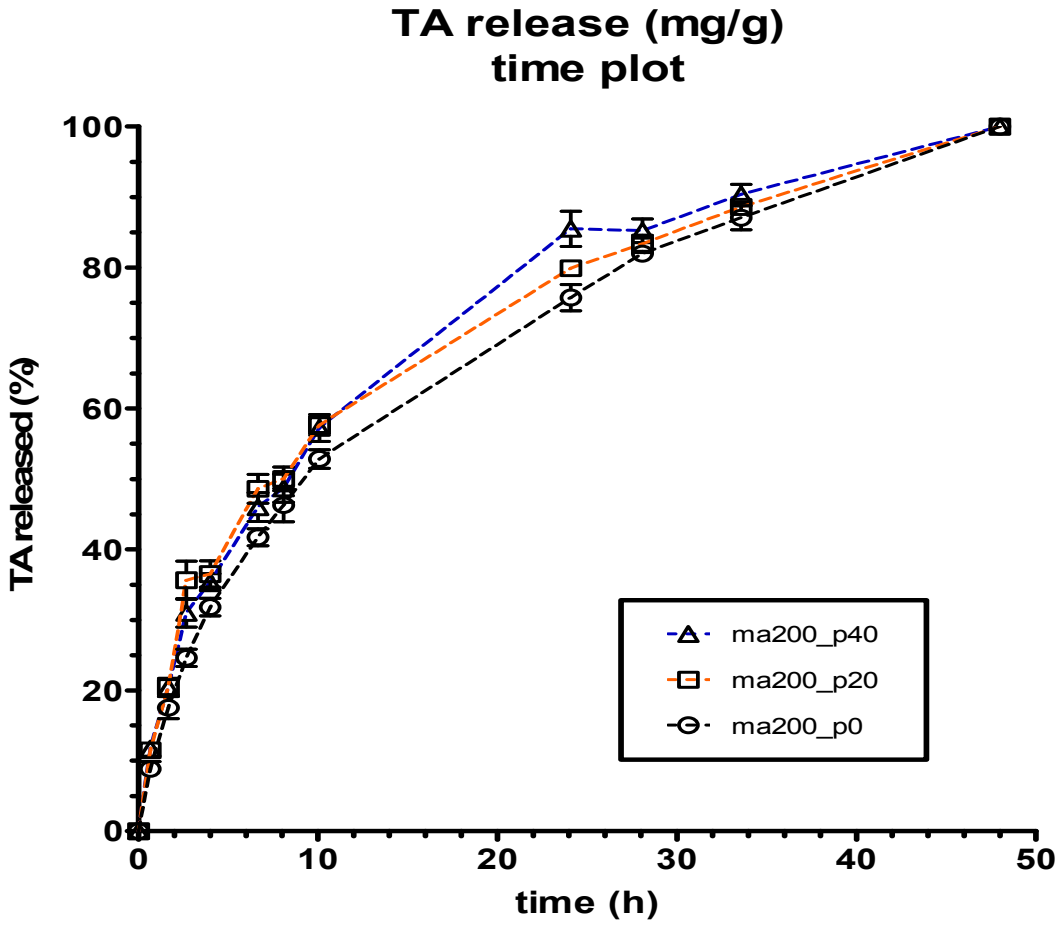


Figure 30: Release of TA from lenses prepared by UV-polymerization process, as obtained in drug release studies.

5 Conclusions

Various soft contact pHEMA based lenses were formulated employing thermal polymerization process, some incorporating MA others NVP. Physiological saline and PBS (pH 7,4) drug solutions were studied as media for drug loading. Studies of swelling showed that lenses behave similarly in physiological saline solution but with the change of solution and pH (PBS with pH 7,4) the MA containing lenses swell in higher degree due to generation of repulsive forces after ionization of -COOH groups. During drug loading studies we discovered that a large fraction of drug binds to the polymer network and that the drug demonstrated equal affinities towards all network types. Loading isotherms had a form of a straight line, a characteristic of the initial stage of Langmuir isotherm, meaning that the binding sites within the hydrogel were far away from being saturated. The loading corresponded well with swelling - it was more efficient in PBS drug solutions than in physiological saline and it was the best with MA200 lenses, up to 0,67 mg/g of lens.

TA release rate in artificial lachrymal fluid was concluded to be solely diffusion controlled. We obtained reproducible release rates that were highest with lenses containing the highest fraction of MA. These released 60% of the dose in the first 8 h and more than 80% in the first 24 h. Based on the results it was possible to conclude that MA200 hydrogels present the best formulation and the PBS drug solution with slightly basic pH is the best option for drug loading.

In order to improve our formulations we incorporated water in the monomer mixtures employing photochemical polymerization processes and obtained lenses of elevated porosity. We chose to evaluate only lenses containing 200 mM of MA and to use only PBS drug solution for drug loading since we had already obtained the best results with this combination. Swelling as well as drug loading was increased with porosity and the highest amount of drug loaded reached 0,79 mg/g in lenses containing 40 vol% of water. The release rate on the other hand was not affected.

We can conclude that our lenses are an appropriate system for controlled delivery of TA. A good way to improve their loading capacity is to improve their porosity. Since the loading isotherms showed that the binding sites were far from being saturated it would be interesting in the future to try to improve drug solubility with the use of nanotechnology.

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