Universidade de Lisboa

Faculdade de Farmácia

Drug Loaded Intraocular Lenses

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Mestrado Integrado em Ciências Farmacêuticas

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Resumo

As cataratas oculares são uma condição patológica que resulta do envelhecimento ou do dano natural do cristalino e que se traduz na respetiva perda de transparência. A opacificação do cristalino provoca a diminuição da visão ou até cegueira, sendo as cataratas a principal causa de cegueira a nível mundial e afetando principalmente os países de médio e baixo desenvolvimento. Esta doença apenas tem uma resolução definitiva, com recurso a uma cirurgia oftálmica, em que se realiza uma pequena incisão na córnea, através da qual é removido o cristalino que se encontra danificado e é colocada no seu lugar, uma lente intraocular artificial. Após a intervenção cirúrgica, os pacientes têm que aplicar colírios anti-inflamatórios e antibacterianos, no olho operado, várias vezes ao dia, durante algumas semanas. A adesão à terapêutica, no pós-operatório, nestes casos é relativamente baixa, por se tratar de um processo moroso que exige destreza. Além disso, a percentagem de fármaco que atinge o alvo terapêutico, é relativamente baixa, devido ao método de aplicação que é propenso a que ocorra uma má utilização. De modo a colmatar estas falhas na adesão do paciente à terapêutica e de modo a obter uma maior biodisponibilidade do medicamento, coloca-se a alternativa de impregnar as próprias lentes intraoculares com fármaco. Estas lentes têm como intuito libertar a substância ativa após a colocação na câmara posterior do olho, durante um período de tempo prolongado, de cerca de uma semana, permitindo ao paciente diminuir a utilização de colírios. Deste modo, pretende-se contornar o problema da fraca adesão à terapêutica e conseguir diminuir o risco de complicações no pós-operatório.

As lentes intraoculares começaram a ser aplicadas em 1949, no Reino Unido, e inicialmente eram fabricadas a partir de polimetil meta-acrilato (PMMA). Com o desenvolvimento dos materiais, as lentes passaram a ser produzidas a partir de materiais acrílicos e podem ser hidrofílicas ou hidrofóbicas consoante os monómeros que as constituem e as proporções em que estes estão presentes na formulação. As lentes hidrofílicas absorvem mais água e como tal, podem ser impregnadas de fármaco através de uma técnica de absorção, que consiste em mergulhar as lentes numa solução que contém o fármaco pretendido, durante um certo período de tempo em determinadas condições ambientais, para que o fármaco seja absorvido. Apesar destas lentes serem bem toleradas pelo olho, estão também associadas a um elevado risco de ocorrência de opacificação da cápsula posterior (OCP). Por outro lado, as lentes hidrofóbicas estão associadas a um menor risco de complicações pós-cirúrgicas e são, atualmente, as lentes mais utilizadas nos países economicamente

mais desenvolvidos. Estas lentes têm uma menor capacidade de absorção de água, pelo que, para serem impregnadas com fármaco, deve ser utilizada uma técnica de revestimento.

Com o objetivo de encontrar os parâmetros ideais para obter lentes intraoculares impregnadas com uma quantidade significativa de fármaco e que libertem esse mesmo fármaco com um perfil adequado e prolongado no tempo, foram realizados vários estudos. Neste sentido, foram executadas as técnicas de absorção e de revestimento em lentes hidrofílicas e hidrofóbicas, respetivamente, fazendo variar diversos parâmetros. As lentes utilizadas nestes estudos foram produzidas por uma técnica de polimerização em massa.

Utilizaram-se lentes acrílicas hidrofílicas em três estudos no âmbito da técnica de absorção. No primeiro estudo, variou-se o tempo de absorção, ou seja, o tempo em que as lentes permaneceram na solução de fármaco, entre 15 e 120 minutos. Foi possível concluir que quanto mais prolongado o período de exposição das lentes à solução, maior a absorção de fármaco. No segundo estudo, alterou-se a quantidade de *cross-linker,* agente essencial para a polimerização, na formulação das lentes. Em teoria, ao aumentar a quantidade de *cross-linker*, a matriz do polímero fica mais condensada e poderá aumentar a capacidade de encarceramento do fármaco, levando a uma libertação mais prolongada. Experimentalmente, verificou-se que quantidades demasiado pequenas de *cross-linker* fazem com que a libertação do fármaco seja praticamente imediata. Por outro lado, concentrações elevadas de *cross-linker* não permitem a absorção de fármaco de um modo eficaz. Assim, conclui-se que uma quantidade intermédia de *cross-linker* será a ideal para atingir um equilíbrio entre absorção e libertação prolongada de fármaco. Por fim, no terceiro estudo, foram utilizadas lentes acrílicas hidrofílicas com monómeros hidrofóbicos na sua constituição, para a absorção de um fármaco hidrofóbico, a teofilina. De modo a testar a absorção deste fármaco, foram utilizadas soluções com diferentes rácios de água e etanol. Foi possível verificar que uma solução com maior quantidade de etanol é mais eficaz para a absorção de teofilina pelas lentes, apesar de uma solução unicamente alcoólica não ser eficaz na absorção de teofilina por parte das lentes.

No estudo de revestimento, foram utilizadas lentes hidrofóbicas. Estas lentes foram revestidas em duas extremidades opostas da superfície lateral, com várias camadas de solução de fármaco e de polímero biodegradável. A solução contendo fármaco foi adicionada primeiro, sendo depois coberta com solução de polímero biodegradável. Este polímero vai-se degradando quando em contacto com o humor aquoso, libertando o fármaco gradualmente, de acordo com uma curva específica para

o polímero em questão, o ácido poli láctico-co-glicólico. Deste modo, foi possível garantir a adição de fármaco a lentes intraoculares sem a alteração das suas propriedades óticas.

Outras técnicas estão a ser desenvolvidas para impregnar lentes intraoculares, apesar de ainda não existirem lentes destas disponíveis no mercado. Além das técnicas de absorção e de revestimento, existem também técnicas que incluem o uso de ciclodextrinas, de lipossomas, de fluídos super-críticos, de nanopartículas, entre outras, que estão neste momento a ser desenvolvidas.

O desenvolvimento destas lentes intraoculares com fármaco incorporado poderá ser um passo importante para o crescimento e desenvolvimento da terapêutica personalizada. Esta tecnologia permite a adaptação das lentes ao doente em questão, alterando o fármaco impregnado ou modificando o perfil de libertação do mesmo. Estas lentes são mais indicadas para doentes idosos que não possuam acompanhamento no pós-operatório e que tenham dificuldade na aplicação frequente dos colírios. Também em doentes diabéticos com maiores riscos de desenvolvimento de complicações pós-cirúrgicas devido à sua patologia base, estas lentes poderão ser mais eficazes, pela atuação local e específica do fármaco. Deste modo, os progressos resultantes da adequação desta tecnologia, poderão ser refletidos numa diminuição da incidência de complicações pós-operatórias a curto e longo termo e, consequentemente, na diminuição dos casos de cegueira.

Palavras-chave: Cataratas; Lentes intraoculares; absorção; revestimento;

Abstract

Ophthalmic cataracts is a disease responsible for visual impairment and loss of vision worldwide, especially in developing countries. The treatment for this condition is chirurgical removal of the natural damaged lens and implantation of an artificial lens to replace it. In post-op, patients need to apply eye drops several times a day, including night-time, during some weeks, to avoid infection and decrease inflammation. This can be an arduous work, especially for elderly people. Thus, the implantation of drug-loaded intraocular lenses could be a beneficial solution to reduce complications after surgery, in cases where the patients´ compliance is diminished.

Intraocular lenses were first discovered in 1949 in the United Kingdom and were made of poly-methyl methacrylate (PMMA). Nowadays, the majority of the lenses are made of acrylic materials and they can be hydrophilic or hydrophobic, according to the monomers used to produce them. These lenses can be drug-loaded by several techniques, like soaking and coating. Hydrophilic lenses can absorb more water; thus, they are prone to be loaded by a soaking technique. On the other hand, hydrophobic lenses do not absorb water in significant amounts. Hence, they need to be loaded by a coating technique. The loading and release of the drug from the lenses vary according to some parameters. So, it is possible to adequate the loading and release profiles according to the patients´ needs.

Drug-loaded intraocular lenses can help the improvement of the patient´s compliance and can also be used to obtain a personalized therapy, if necessary. Above all, it is intended that this technology, by releasing medicine closer to the target, will decrease the post-surgery complications and reduce the incision of secondary cataracts or other pathologies related to the surgery.

Keywords: Cataracts; Intraocular lenses; Soaking; Coating;

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I would like to thank my tutors and my parents to help me through this work, specially to Marius Heck, for all his help since my stay in Berlin. I would also like to thank my friends for all the doubts shared and help when in need.

Abbreviations

IOL- Intraocular lenses

PCO- Posterior Capsule Opacification

NSAIDs- Non-steroid anti-inflammatory Drugs

HEMA- Hydroxyethyl methacrylate

MMA- Methyl methacrylate

PMMA- Poly-methyl methacrylate

EDMA- Ethylene dimethacrylate

BP- Benzoyl Peroxide

API- Active Pharmaceutical Ingredient

PLGA – Poly Lactic-co-Glycolic Acid

POEA - Phenoxiethyl acrylate

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1 Introduction

According to the World Health Organization (WHO) it is estimated that 285 million people worldwide are visually impaired, meaning they either are blind or have low vision. A lot of causes can lead to impaired vision. Among these, cataracts are the leading cause of blindness in middle and low income countries (1).

A cataract is an opacity of the natural lens of the eye that causes partial or total blindness. This condition usually appears in elderly people due to the degenerative effects of aging on cell structure but there are also a variety of risk factors that can lead to the development of cataracts (2). Nowadays, the only working treatment for this condition is surgery where the natural damaged lens is removed and an artificial lens is inserted in its place, called intraocular lens (IOL). These lenses may have correction for large refractive errors improving the patient´s vision not only by the addition of a new transparent lens but also by correcting other previous problems that might exist (3).

Intraocular lenses started to be used in the 20th century in London by Sir Harold Ridley, who discovered that polymethyl methacrylate (PMMA) pieces were inert in Royal Air Force (RAF) pilots´ eyes, injured during flights in the second World War. The first cataract surgery introducing an artificial IOL was performed in 1949 in the United Kingdom (3,4).

In Portugal, it is estimated that the number of cataract surgeries increased from around 14.000 in 1993 to 147.000 in 2009. With the aging of the population the incidence and prevalence of cataracts will continue to increase. Consequently, the number of surgeries will rise (5).

Cataract surgery starts by a small incision in the cornea. After, the opaque lens is aspirated by a probe and an IOL is inserted in its place (Figure 1).

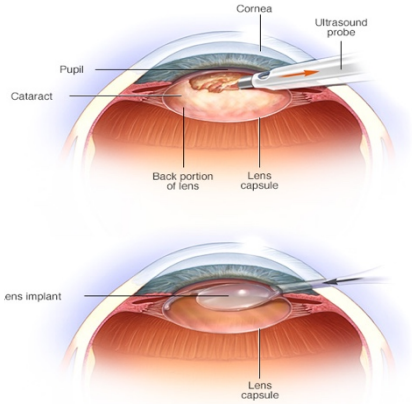

Figure 1: Cataract surgery (6)

To stay fixed in the eye IOLs have some small hooks to keep the lens in place once inserted in the eye, which are called *haptics* (Figure 2).

Figure 2: IOL with haptics (7)

To avoid major problems like infection and to decrease the inflammation after the surgery patients need to apply antibiotic and corticosteroids or nonsteroidal anti-inflammatory drugs (NSAIDs) in the form of eye drops several times a day for four to eight weeks. Applying eye drops is a distressing work that can reduce patients´ compliance. Not only it implies frequent care by the patient but there is also a high probability that the eye drops are incorrectly applied, causing loss of the medicine. Besides, eye drops already have a low eye targeted bioavailability, of around 1% to 10%. Thus, the creation of drug-loaded intraocular lenses could allow the reduction of eye drops applications, increasing the patients´ compliance and the quantity of drug that reaches the target.

1.1 Intraocular Lenses (IOLs)

The first IOLs attempted to be used, in the 18th century, were made of glass (8). Afterwards, they started to be made of polymethyl methacrylate (PMMA) which is a hydrophobic material, rigid and non-foldable. Silicon is also used as lens material since 1984. It is an extremely hydrophobic material which gets very slippery when wet (4). Also, these lenses unfold very fast which can damage the eye. Consequently, silicon lenses are almost not used anymore (<1%). Nowadays, in developed countries acrylic hydrophobic lenses are the most common ones because they lead to less complications in post-surgery and are easy to handle. However, in developing countries, hydrophobic polymethyl methacrylate (PMMA) lenses also known as Perspex or Plexiglas, continue to be the ones used due to economic restrictions and because they are well investigated although they don´t have the best profile.

Nowadays, lenses can be made of a variety of materials depending on the

patient and doctor´s preferences. While the surgeon searches the easiness of implantation and lack of intraoperative complications, the patient asks for long lasting lenses with refractive stability (3). Drug loading of these lenses can be done by several techniques according to the drug used and the lens´ nature. All in all, it is important to achieve a prolonged drug release. Long enough to help the eye to restore its properties but not long enough to cause eye damages, like adverse effects in healthy tissues or systemic absorption.

Over time, IOLs can have some problems like swelling due to the absorption of the aqueous humour absorption. The aqueous humour is a clear and colourless fluid that fills the anterior chamber of the eye. It is composed of proteins, electrolytes and cytokines and it plays an important role in keeping the intraocular pressure and providing nourishment and nutrients to the eye cells. Its pH is around 7.2 – 7.5 (9). This liquid absorption can form small vacuoles on the lens which are called glistenings and can change the optical properties of the lens (4). Lenses can be displaced if the haptics run out of place, possible injuring the eye´s tissues. Moreover, the lenses can turn opaque due to creation of biofilms on their surface. Also the posterior side of the natural lens´ capsule can turn opaque after surgery which is named Posterior Capsule Opacification (PCO) and can lead to a second cataract (8). These problems may depend on the lens material, on the Active Pharmaceutical Ingredient (API) itself and on the surgical technique and some may be avoided by the drug loading of the IOL.

Drug loading and release from these lenses are influenced by several factors like the nature of the lenses´ materials, the affinity of the drug to the lens and to the water, among others. Also, the optical properties of the lenses may be changed by the drug loading method, the material of the lenses and the drug itself. The lenses can be characterized as hydrophilic or hydrophobic according to the angle made by a drop of water in the lens´ surface.

Hydrophilic acrylic lenses are very resistant and somewhat compressible, what makes it easier to insert in the eye with a smaller incision (<2 mm), since they can be inserted in a foldable way and unfold once in place. Also, these lenses are not sticky, so they do not need lubricant, once applied. On the other hand, they must be delivered to the surgeon in a wet state or they will turn hard and will become brittle. With loaded lenses, this can be a problem for the producer as it raises stability issues to deliver the lenses in a wet state. Besides, these lenses are related to a higher percentage of PCO cases. Hydrophobic lenses are prone to damage by the surgical materials but they can be delivered in a dry state and have better optical properties, like a higher refraction index (4). Their application needs to be followed by the use of lubricant because these

lenses tend to get a sticky consistence. Nowadays, hydrophobic acrylic lenses are the most used ones, in developed countries.

1.2 Drug Loading

1.2.1 Soaking Technique

This method is the simplest and more cost-effective. It consists in soaking the IOLs in drug solutions for a certain period of time allowing the drug to penetrate the lenses. The drug loading capacity of the lens is affected by some factors like the lens´ water content and thickness, the molecular weight of the drug, the soaking time in the drug solution and the concentration of this solution (10). The lenses work like a reservoir for the drug. Hence, the drug needs to have affinity to the lens´ polymers to be absorbed. So, if the polymer is hydrophilic, the drug should be water-soluble, in order to reach a higher absorption.

It is known that with an increase in the hydrophilic phase there is an additional increase in the drug loading. On the other hand, an increase in the hydrophobic phase will promote a sustained release (10). Thus, it is interesting to load lenses with a higher quantity of hydrophobic monomers so the patients can benefit from a more prolonged release. In theory, the loading of these lenses can be more effective using organic solvents like ethanol.

Though the advantages of this method, it depends on the molecular weight of the drug which proofs to be inadequate for some drugs. If the molecular weight of the API is too high, it will not be able to enter the polymers´ matrix and the lenses will not soak any drug.

1.2.2 Coating Technique

Intraocular lenses may change their optical properties when drugs are incorporated into the lens matrix. This problem is especially prominent in hydrophobic IOLs. Therefore, the coating technique allows the loading of acrylic hydrophobic lenses which have a low water content.

This method consists in covering the lateral surface of the IOLs with a drug solution. A biodegradable polymer is added to the drug solution to achieve a sustained release of the API. This biodegradable polymer, named Poly Lactic-co-Glycolic Acid (PLGA) will work as a glue to attach the API solution to the lens. At the same time, it

will act as a retarding agent since it works as a cover layer that will disintegrate along time and will uncover the drug which will, in turn, start its release.

1.3 Purpose

In the work performed, according to the experiment and its specific purpose, more hydrophilic or hydrophobic lenses were used. IOLs can have different proportions of hydrophilic and hydrophobic components. Hydrophilic lenses will have better API soaking capacity but hydrophobic lenses will have better API releasing profiles in the aqueous humour. Hydrophilic IOLs can be drug-loaded by soaking due to the high-water content. On the other hand, hydrophobic IOLs have a lower water content so it is more difficult to drug-load them by soaking and the release occurs via diffusion coefficient, which makes it more prolonged. Therefore, they might not achieve the therapeutic window. Thus, these lenses need to be drug-loaded by alternative techniques like API coating, for example.

Furthermore, some drugs have a higher molecular weight or have low affinity for the lenses materials so they only get attached on the surface and suffer a burst release, not attaining the therapeutic window. In these cases, soaking method can be replaced by a coating method.

Considering what was previously mentioned, one of the major issues regarding IOLs is getting a prolonged release of therapeutic concentrations of API in an intraocular lens that keeps its optical properties intact through time. Therefore, the main objective of this work was to study the optimum conditions and factors necessary to achieve a prolonged therapeutic release while avoiding the problems that may appear. In order to do this, different lens´ materials were used and loaded with diverse drugs by two different techniques, either soaking or coating. By improving these drug-loaded IOLs it is intended to obtain lower rates of post-operatory complications of cataract surgeries and to improve patients´ quality of life over time.

2 Materials and Methods

2.1 Intraocular Lenses (IOLs)

During the work developed, different lenses were produced via bulk polymerization, according to different formulations (Table 1). Intraocular lenses have around 0.6 mm of center-thickness and 6 mm of diameter with an average weight of 20 mg.

Table 1: IOLs formulations: Monomers ratio

Formulation	Monomers	Mass (g)
1	HEMA : EDMA : BP	(9.95 : 0.05 : 0.05)
2	HEMA : MMA : EDMA : BP	(7.95 : 1,.95 : 0.05 : 0.05)
3	POEA : EDMA : BP	(9.95 : 0.25 : 0.25)
4	MMA: EDMA: BP	(9.95 : 0.05 : 0.05)

2.1.1 Hydrophilic Acrylic IOLs

The lenses produced with hydrophilic materials have a higher water uptake, around 19-26% (4). They have a contact angle with water lower than 50º and a refractive index of 1.43.

Hydroxyethyl Methacrylate (HEMA) (Merck KGaA, Darmstadt, Germany) was one of the materials used to produce hydrophilic IOLs (Figure 3), (Table 1) The monomers can be toxic. After the polymerization, the polymers are inert but some monomers´ residues can be present. So, the polymers undergo a purifying step with ethanol to remove these residues.

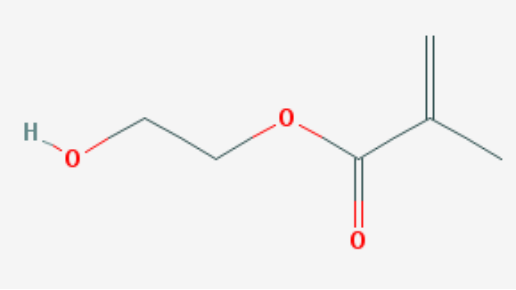

Figure 3: Hydroxyethyl Methacrylate (HEMA) (11)

2.1.2 Hydrophobic Acrylic IOLs

Hydrophobic acrylic lenses can retain 0.05-2% of water inside the matrix. The refractive angle can vary between 1.44 and 1.55 (4).

Methyl Methacrylate (MMA) (Merck KGaA, Darmstadt, Germany) is a hydrophobic monomer which was also used to produce the polymers (Figure 4) (Table 1). It can be used alone forming PMMA polymers or in combination with HEMA to produce hydrophilic or hydrophobic polymers according to the percentage of each one used.

The refractive index of PMMA lenses is around 1.49 and they can be delivered in a dry state. There is the disadvantage of being very rigid (4).

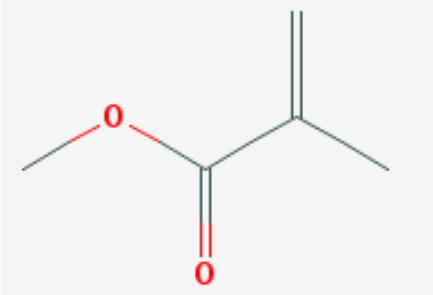

Figure 4: Methyl methacrylate (MMA) (12)

Phenoxyehtyl acrylat (POEA) (Merck KGaA, Darmstadt, Germany) is an acrylic hydrophobic material which was also used to produce soft lenses (Figure 5).

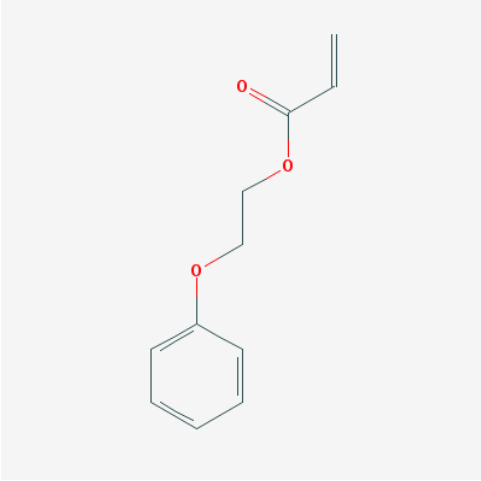

Figure 5: Phenoxiethyl acrylate (POEA) (19)

2.1.3 Artificial Aqueous Humour

A solution of phosphate buffer with pH=7.21 was prepared to simulate the aqueous humour (Table 2). The components, potassium dihydrogen phosphate (Carl Roth GmbH + Co Kg, Karlsruhe, Germay) and Di-sodium hydrogen phosphate dihydrat (Merck KGaA, Darmstadt, Germany), were mixed in an Erlenmeyer combined with 5 litters of purified water. The mixture was stirred in a magnetic plate with an electromagnetic stirrer until total dissolution. Afterwards, the pH of the buffer was measured using a Metrohm pH meter (Metrohm Ltd., Herisau, Switzerland). This artificial aqueous humour was used as a release medium for the experiments, to simulate the pH of the posterior chamber of the human eye.

Table 2: Artificial aqueous humour formulation

Composition	Weight (g)
KH_2PO_4	13.495 g
Na_2HPO_4 .2 H_2O	42.452 g

2.2 Bulk-Polymerization

All the IOLs were produced via bulk-polymerization. This technique consists in joining pure monomers or a mixture of monomers to an initiator and a cross-linker and is usually used when the aim is to obtain a clear polymer. By adding heat the reaction will start and the monomers will connect and form a dense matrix. This method allows the production of transparent polymers.

Ethylen glycol dimethacrylate (EDMA), (Merck KGaA, Darmstadt, Germany), was used as cross-linker and benzoyl peroxide (BP), (Merck KGaA, Darmstadt, Germany), as a thermal initiator. BP undergoes symmetrical fission, forming two benzoyloxy radicals (Figure 6). The radicals will react with the monomers and with the cross-linker, creating polymer chains.

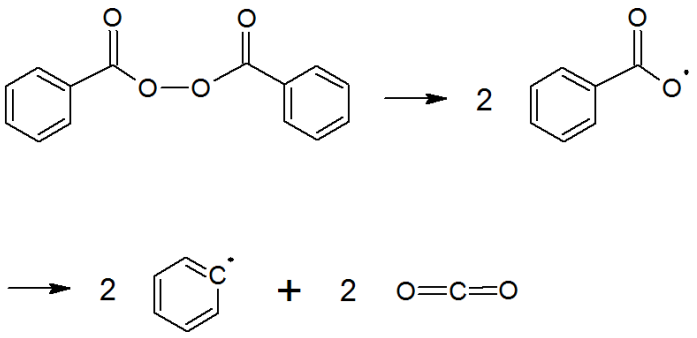

Figure 6: Benzoyl Peroxide mechanism of action (13)

The liquid compounds were mixed in a magnetic stirrer (IKA®-Werke GmbH & Co KG, Staufen, Germany) and degassed for 10 minutes by purging with N_2 gas to remove the oxygen and prevent the formation of air bubbles inside the polymers. The mixture was placed in Eppendorf tubes and the polymerization was performed out in the oven (Heraeus T6030, Hanau, Germany) at 50°C for 72 hours. The solidified polymers were pulled out of the tubes and stored in ethanol for 24 hours to remove remaining monomers, initiator and cross-linker. This was named the purifying step. Afterwards, the organic solvent was removed in a vacuum oven (Heraeus VT 5042 EKP, Hanau, Germany) coupled with a chemistry hybrid vacuum pump (Vacuubrand GmbH, Wertheim, Germany) at room temperature for 96 hours. Then, the polymer rods were sliced into small lenses, polished and they were ready to use. If the polymers were not used right away, they were kept in glass vials with purified water. This technique was applied in all the lenses used for the experiments.

2.3 Drugs

Table 3: API Information

API	Origin	Water Solubility	UV Absorption
Diclofenac Sodium	BASF, Ludwigshafen, Germany	1.50 g/L (14)	276 nm
Propranolol Hydrochloride	K.-W. Pfannenschmidt GmbH, Hamburg, Germany	50.0 g/L (15)	212, 289, 319 nm
Theophylline anhydrous powder micronized	BASF, Ludwigshafen, Germany	7.36 g/L (16)	272 nm
Dexamethasone	Fagron GmbH, Barsbüttel, Germany	0.089 g/L (17)	242 nm

All the materials and equipments were purchased by the Department of Pharmaceutical Technology of the Faculty of Pharmacy of the *Freie Universität, Berlin*.

2.4 Soaking Technique

2.4.1 Soaking Times

Formulation 1 (Table 1) lenses were produced by bulk polymerization. Five batches made of three lenses each were prepared. Every batch was weighted in a Balance Acculab Vic-303 (Sartorius Group, Goettingen, Germany) and placed in test tubes. Four of the test tubes contained a soaking solution of diclofenac sodium 0.5% and the fifth tube was used as the blank. The lenses remained in the solution for 15min, 30min, 60min and 120min (n=4). Afterwards, the lenses were removed from the test tubes with forceps and they were washed with some drops of purified water to remove the excess of soaking solution. Subsequently, the lenses were gently dried with absorbent paper to remove any remains of the diclofenac solution. The refractive index was analyzed by Abbe Refractometer (Carl Zeiss AG, Oberkochen, Deutschland). Next, each batch of lenses was weighted again and placed in cleaned test tubes containing 4mL of artificial aqueous humour (pH= 7.21). The tubes stayed in the incubation shaker (New brunswick scientific GmbH, Nürnberg, Germany) with a shaking rate at 100 rpm, at 37ºC. At specific times, the whole media was exchanged and analyzed by UV spectroscopy (Agilent HP 8453, Agilent Technologies Inc., Santa Clara, US) at 276 nm until no more drug release was verified.

2.4.2 Cross-linker amounts

In this experiment, different formulations (n=5) of IOLs were produced by changing the amount of cross-linker, EDMA (Table 4).

Table 4: Different cross-liker amounts´ formulations

Formulation 1	HEMA : EDMA : BP	(9.95 : **0.05** : 0.05)
Formulation 1 A	HEMA : EDMA : BP	(9.95 : **0.01** : 0.05)
Formulation 1 B	HEMA : EDMA : BP	(9.95 : **0.1** : 0.05)
Formulation 1 C	HEMA : EDMA : BP	(9.95 : **0.15** : 0.05)
Formulation 1 D	HEMA : EDMA : BP	(9.95 : **0.5** : 0.05)

After the removal of the organic solvent by vacuum oven, each formulation polymer rods were placed in different glass vials with purified water for a week to become more flexible. Next, the rods were cut in lenses and three lenses of each of the 5 formulations were weighted and placed in different glass vials with 5mL of soaking solution, propranolol hydrochloride 1%, for 2 hours. Afterwards, the lenses were removed from the test tubes with forceps and they were washed with some drops of purified water to remove the excess of soaking solution. Subsequently, the lenses were gently dried with absorbent paper to remove any remains of the propranolol hydrochloride solution. Next, each batch and each lens was individually weighted and placed in cleaned test tubes, one lens in one tube, containing 4mL of artificial aqueous humour (pH= 7.21). The tubes stayed in the incubation shaker with a shaking rate of 100 rpm, at 37ºC. At specific times, the whole media was exchanged and analyzed by UV spectroscopy at 212, 289 and 319 nm until no more drug release was verified. The refractive index was also analyzed by Abbe Refractometer during the release.

2.4.3 Soaking solution with organic solvent

Formulation 2 (Table 1) lenses prepared by bulk polymerization and stored in a glass vial immersed in water, were used. Seven batches of five lenses each were prepared. The batches were weighted individually and placed in glass vials. Each batch was soaked in a different theophylline 0.5% soaking solution with different proportions of water and ethanol (Table 5). The seventh batch was used as blank and was soaked in water.

Table 5: Theophylline soaking solutions

	Water (mL)	Ethanol (mL)	Theophylline (g)
A	50	0	0.2525
B	40	10	0.2580
C	30	20	0.2546
D	20	30	0.2511
E	10	40	0.2521
F	0	50	0.2554
Blank	50	0	0

After 24 hours in the soaking solutions, the lenses were removed from the vials, gently dried and weighted by batch. Afterwards, they were placed in the vacuum-oven for four days to remove the remaining ethanol. While in the vacuum-oven, the lenses were weighted from time to time until constant weight. When removed from the vacuum-oven, the lenses were weighted again, individually. Next, each lens was placed in a test tube containing 4 mL of artificial aqueous humour (pH= 7.21). The tubes stayed in the incubation shaker with a shaking rate of 100 rpm, at 37ºC. At specific times, the whole media was exchanged and analyzed by UV spectroscopy at 272 nm until no more drug release was verified. Transmittance was checked in the range of 300 – 850 nm, to verify the transparency of the lenses at the visible wavelength.

2.5 Coating Technique

Homopolymers for IOLs were prepared by bulk polymerization of methyl methacrylate (MMA) and phenoxy ethylacrylate (POEA) to obtain hard and soft intraocular lenses, respectively (Table 1).

Two 1 mL coating solutions were prepared with a mixture of 5.0% of dexamethasone and 0.5% of PLGA 502 A (Evonik Industries AG, Darmstadt, Germany) and a pure 5.0% PLGA 502 A solution, respectively. The dexamethasone and PLGA 502A powders were weighted in a Mettler Toledo MX5 microbalance, (Mettler Toledo, Greifensee, Switzerland), to get precise measures of the small quantities and afterwards, they were dissolved in ethyl acetate (Table 6).

Table 6: Composition of the coating solutions

	PLGA 502 A (g)	**Dexamethasone (g)**
Sub-coating	0.005187	0.050400
Top-coating	0.050157	-

The coatings were applied layer by layer on the lateral surface, punctual at the edges or at two sides of the lenses with the help of an insulin needle (Figure 7). Later, the first coating solution was used as sub coating and the pure PLGA solution as top coating (Figure 7).

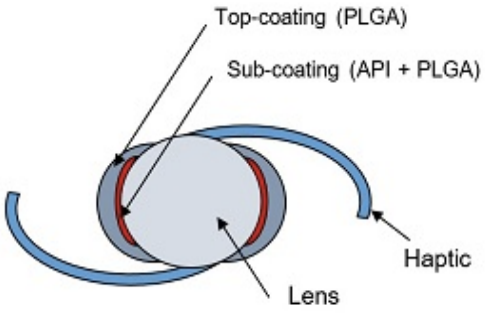

Figure 7: Coated IOL

Different layers of dexamethasone and PLGA were applied to the PMMA lenses and POEA lenses (3-A; 3-B; 3-C), (Table 7). After the application of each layer a waiting time was necessary to guarantee that the applied layer would be dry before the application of the following layer. After the application of all the layers, the lenses remained in the hotte followed by vacuum-oven over-weekend, to ensure complete dryness of the layers.

Table 7: Proportion of layers

Batch	5.0% Dexamethasone (mg) + 0.5% PLGA (mg)	5.0% PLGA 502 A (mg)	Lenses material
1	1	0.10	PMMA
2	1	0.35	PMMA
3	1	0.60	PMMA
4	1	0.85	PMMA
5	1	1.1	PMMA
3-A	0.5	0.60	POEA
3-B	1.5	0.60	POEA
3-C	1	0.60	POEA

Afterwards, each lens was placed in a glass vial containing 8 mL of artificial aqueous humour (pH= 7.21). The vials were kept in the incubation shaker at 37°C and at a shaking rate of 100 rpm. At specific time points, the whole media was refreshed and samples were collected and analyzed by UV spectroscopy at 242 nm. Also, the

pH of the release medium was measured at some sample points. The refractive index was analyzed by Abbe refractometer and transmittance was checked in the range of 300 – 850 nm. Photos of the lenses were taken before, during and after the release with a digital microscope (DigiMicro Lab 5.0, dnt Drahtlose Nachrichtentechnik, Dietzenbach, Germany).

2.6 UV Spectroscopy

In all the experiments, the release medium was frequently changed and immediately analysed by UV spectroscopy at specific wavelenghts according to the absorbance of the API (Table 3) until the end of the release process. Due to a high concentration, some solutions had to be diluted in order to stay within the limits of detection of the UV Spectrometer.

A calibration curve was made for every used API. With the absorptions obtained by the UV spectrometer it was possible to calculate the amount of API released by each lens and consequently, previously soaked. These calculations were done via a linear regression approach.

The transmittance of the lenses was also measured at the UV Spectrometer in the range 300-850, which corresponds to the visible wavelength. After the release was concluded, the lenses were placed in the spectrometer and analysed.

3 Results and Discussion

3.1 Soaking Technique

3.1.1 Soaking Times

After calculating the drug uptake for a standard 20 mg lens, it was possible to observe that the drug uptake was higher in the batch that spent more time in the soaking solution. The lenses that were kept 15 minutes in the soaking solution had a low drug uptake (71% less uptake, i.e., comparison between 15 min and 120 min soaking time) (Table 8).

Table 8: Drug uptake of different soaking times

Soaking Time (min)	Average weight (g)	Drug Uptake (ug/lens)
120	0.03923	67.71 ± 8.76
60	0.02753	57.20 ± 5.17
30	0.03277	44.63 ± 0.30
15	0.03180	19.28 ± 0.83
0	0.02480	-

Following the soaking step, the refractive index from a lens of each batch was measured by an Abbe Refractometer (Table 9). It is possible to conclude that the soaking of the API did interfere with the optical properties of the lenses, since the refractive index decreases with the increasing time in the soaking solution.

Table 9: Refractive index of lenses with different soaking times

Soaking Time (min)	Refractive index
120	1.4520
60	1.4526
30	1.4555
15	1.4621

0	1.4655

The releasing profile was similar for all the soaking times (Figure 8). Diclofenac underwent a burst release; meaning that most of the API was released in a high amount as soon as the lenses were placed in the release medium. Afterwards, the release was controlled by the diffusion coefficient (D), (Figure 8). In these experiments, the flux and the changes of concentration between the lenses and the artificial aqueous humour are given by Fick´s Second Law (Figure 9).

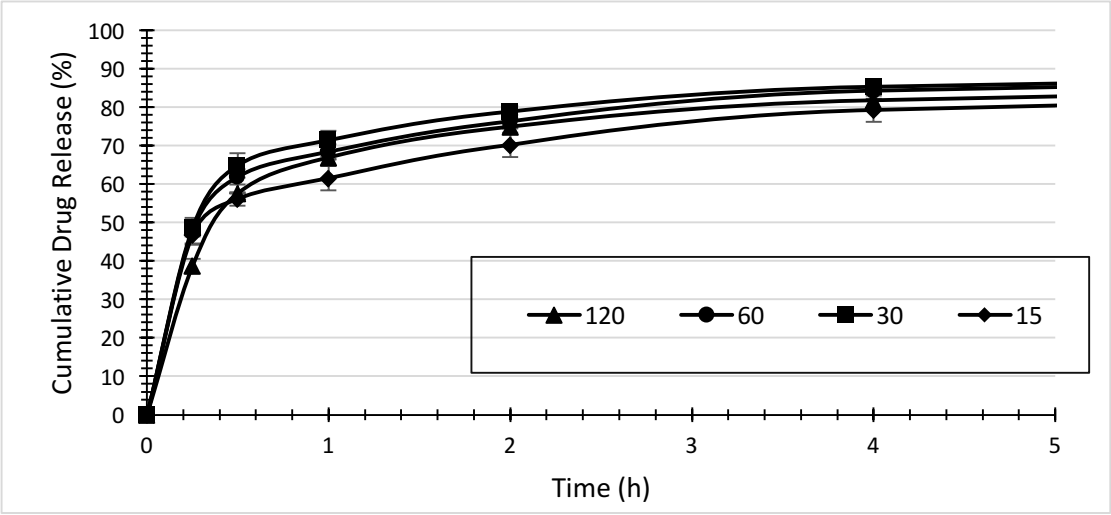

Figure 8: Releasing profile of diclofenac under the influence of different soaking times

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}.$$

$\frac{dc}{dt} = flux$

$D = Diffusion\ coefficient$

$\frac{d^2c}{dx^2} = concentration\ gradient$

Figure 9: Fick´s second law (18)

With this profile, it is possible to conclude that the time spent in soaking solution does not influence the release profile.

Diclofenac sodium was the API chosen for this experiment due to its characteristics. It is an anti-inflammatory drug which makes it a model drug for the IOL. Besides, it is soluble in water.

Like it was expected, it was possible to conclude that the soaking time only influences the amount of API absorbed by the lens but it does not change the releasing profile, neither the optical properties of the IOLs. Since the lenses used were hydrophilic, the more prolonged the time spent in the aqueous medium, the more solution was absorbed, which consequently lead to an increase of the amount of soaked drug. Thus, it is possible to load IOLs with a relevant amount of API if the lenses stay immersed during a reasonable amount of time.

3.1.2 Cross-linker amounts

At the end of the soaking in purified water step to increase the flexibility, it was verified that the formulation with the higher amount of cross-linker was more rigid and difficult to cut, while the other formulations had a good consistency. In all formulations was possible to observe a weight increase after the soaking in the propranolol hydrochloride solution for 2 hours, following the same pattern (Table 10). Hence, with this experiment, it was not possible to reach a conclusion about the influence of the quantity of cross-linker in the capacity to soak water.

Table 10: Weight difference and drug release of lenses with different cross-linker amounts

Cross-linker %	Weight pre-soaking (g)	Weight after soaking (g)	Drug Release (ug/lens)
0.1%	0.3313	0.3355	1038.9 ± 56.4
0.5%	0.2908	0.3000	1483.0 ± 37.2
1.0%	0.3190	0.3295	1176.6 ± 55.8
1.5%	0.2918	0.2962	1249.7 ± 41.1
5.0%	0.2093	0.2140	999.4 ± 47.1

After the end of the release, it was reached the conclusion that the formulations with less quantity of cross-linker had a higher drug uptake (formulations 0.5% and

1.0%). This may had happened because the polymer produced with fewer cross-linker amount has a less condensed matrix. Hence, the API particles are prone to enter inside the matrix and stay confined in there. However, if the matrix has a too few amount of cross-linker, it will not be compact enough and it will not confine the particles, as it happened with formulation 0.1%. On the other hand, if the amount of cross-linker is too high, the matrix will be too condensed and the drug molecules will have more difficulty to penetrate it. This is possible to observe in Table 10, where the formulation with 5% of EDMA had the lowest amount of propranolol hydrochloride released.

The releasing profile of propranolol hydrochloride from the lenses was similar for every formulation (Figure 10). Therefore, it is not possible to conclude that the polymer´s conformation, altered by the amount of cross-linker, changes its releasing properties, although it can change the absorption properties.

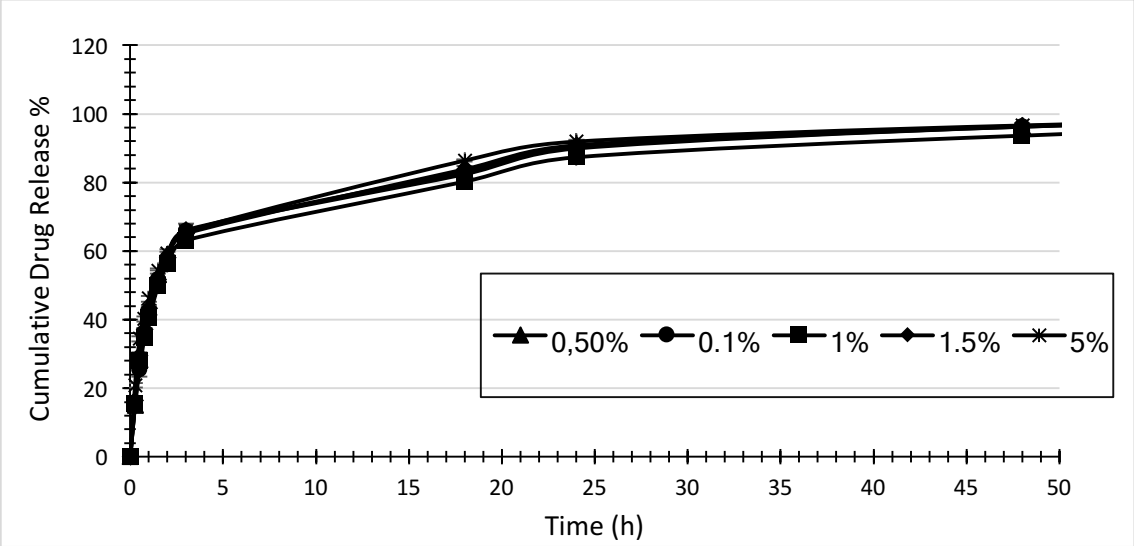

Figure 10: Cumulative propranolol hydrochloride release of lenses with different cross-linker amounts

The optical properties of most the lenses remained unchanged after the soaking and after the release. The lenses were clear and transparent. Besides, the refractive index after soaking did not change appreciably considering the normal value for these lenses formulation, 1.43. Apart from the batch with 5.0% of cross-linker, which had a refractive index of almost 1.45, due to a smaller amount of water in the matrix (Table 11).

Table 11: Refractive index of different cross-linker % lenses

Cross-linker %	Refractive index

0.1%	1.4288
0.5%	1.4260
1.0%	1.4300
1.5%	1.4335
5.0%	1.4480

In this experiment, it was intended to have a highly water soluble model drug, so that the small uptake differences could be perceived. That´s why the chosen API was propranolol hydrochloride. Due to its characteristics, the drug is released quite fast and within 4 days, the release was complete.

Thus, it was possible to conclude that the amount of cross-linker in the formulation shall be between 0.1% and 0.5% in order to get the highest loading of hydrophilic API. Nevertheless, the batches were too small, so it was not possible to reach any significant conclusion regarding the relation between the amount of cross-linker and the water uptake.

3.1.3 Soaking solution with organic solvent

In the soaking solution F, composed only of ethanol and theophylline 0.5% (Table 5), precipitates of theophylline were found. Because theophylline is only slightly soluble in ethanol, at this 0.5% concentration it was not possible to solubilize the API, not even when using the sonification bath.

After the soaking step, the lenses were placed in the oven to remove the remaining ethanol, at room temperature to avoid degradation of the API. The weight of the lenses after four days in the oven was inferior to the weight of the lenses before the soaking step (Table 12). This may have happened because the lenses also have water in their constitution and ethanol and water evaporate simultaneously. Moreover, after the drying step, the lenses become hard and brittle. This confirms the loss of water, which works a plasticiser agent in the polymers.

The lenses that released more theophylline were batches D and E (Table 5). These batches had a higher quantity of ethanol than of water. Consequently, it is possible to conclude that the presence of ethanol will improve the uptake of theophylline. However, batch F which only contained ethanol in the soaking solution did not have a good theophylline absorption. This might have happened because

theophylline was not soluble and the solution concentration was inferior to 0.5%. Thus, it is possible to conclude that the presence of water in the soaking solution is necessary for uptake of API in formulation 2 lenses (Table 1).

Table 12: Weight of each batch before and after soaking and after the drying step

Batch	Weight pre-soaking (g)	Weight immediately after soaking (g)	% of Soaking (%)	Weight after the drying step (g)	Drug Release (ug/mL/lens)
A	0.1415	0.1425	0.1	0.1094	0.83 ± 0.03
B	0.1524	0.1901	3.8	0.1269	0.84 ± 0.03
C	0.1368	0.2575	12.1	0.1151	0.89 ± 0.01
D	0.1383	0.3448	20.6	0.1130	1.22 ± 0.05
E	0.1389	0.3390	26.0	0.1132	1.18 ± 0.16
F	0.1396	0.2262	8.7	0.1142	0.84 ± 0.04
Blank	0.1421	0.1417	0	0.1105	-

The release profile obtained, allows the conclusion that lenses soaked in aqueous solution (solution A), have a more prolonged release comparing to lenses soaked in ethanol (solution F), which had a burst release (Figure 11). This happens because the artificial aqueous humour is an aqueous solution. Hence, lenses soaked with solution A will release the API by diffusion coefficient, meaning that the water goes from the less concentrated to the more concentrated part, creating a flux that will release the API from the lens into the solution, progressively. On the other hand, the lenses soaked with solution F will release their API immediately, because propranolol hydrochloride has a high-water solubility and when in contact with an aqueous solution, it is dissolved immediately. The soaked quantity was very small, so the release was finished in 24 hours.

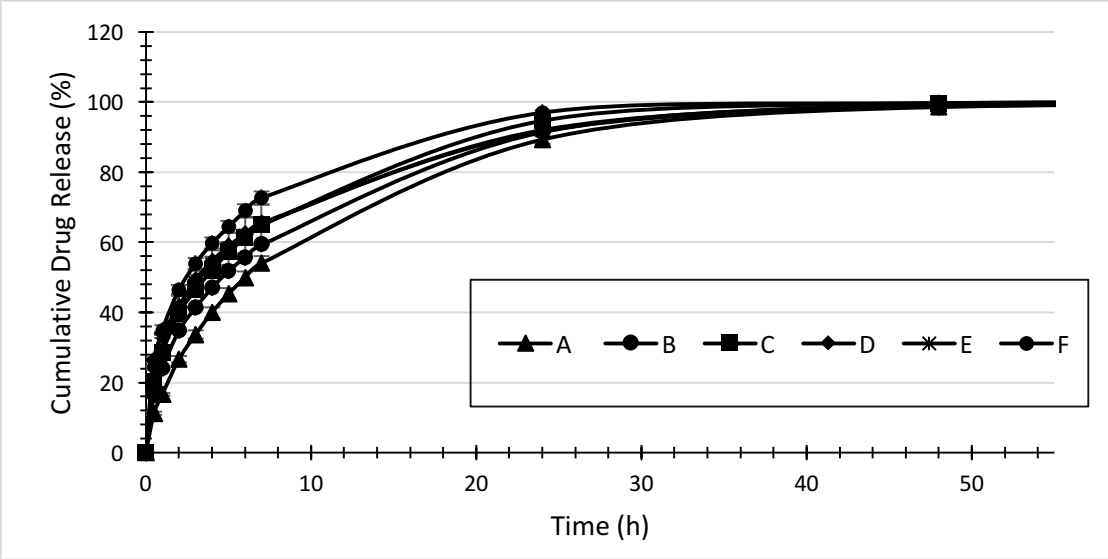

Figure 11: Cumulative theophylline release of lenses soaked in ethanol solutions

The optical properties of the lenses remained intact and a transmittance of 100% was achieved between 350-850nm (Figure 12). Formulation D seems to have 100% transmittance at the entire wavelength spectrum. This may have happened due to a misplacement of the lens in the UV spectrometer.

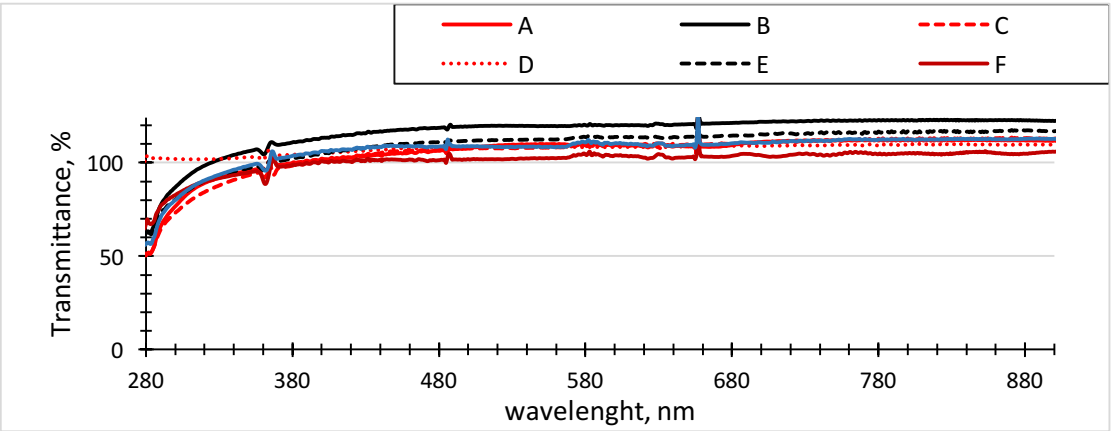

Figure 12: Transmittance of lenses soaked in ethanol solutions of theophylline

With this experiment, it was possible to conclude that for poorly hydrophilic APIs it is better to load the lenses in a mix solution of water with an organic solvent like ethanol. With the right proportion of solvents, it is possible to have a reasonable drug loading and a prolonged release.

3.2 Coating Technique

During the investigation, all lenses showed 100% light transmittance in the range of 300 – 850 nm. The refraction index (pMMA: 1.4900; pPOEA: 1.5565) was also not affected by the coating and did not change during release studies. Due to the lipophilic structure of the polymers, a small amount of water uptake was observed (pMMA: 2.0%; pPOEA: 1.0%). The coating adhesion was good on both formulations but a detachment was observed in the lenses coated at the whole lateral surface (Figure 13).

Figure 13: Detachment of Coating from PMMA lens (lens was not polished)

After placing the lens in the release medium, the PLGA started to swell and to degrade, allowing the release of the dexamethasone (Figure 14).

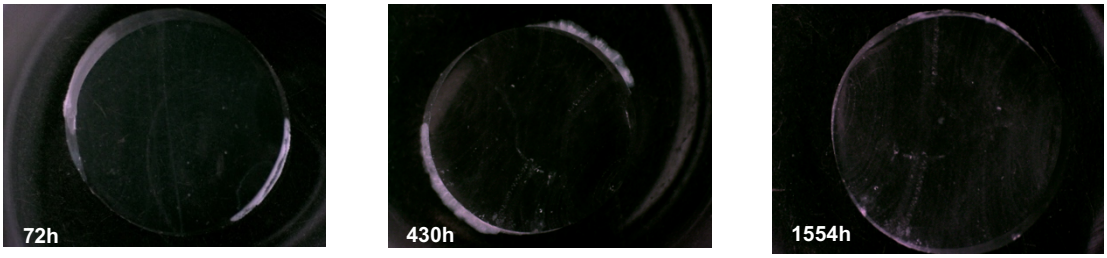

Figure 14: Swelling and degradation of PLGA coated IOLs along time

Along with the degradation of PLGA, acid compounds were formed leading to a decrease of the pH of the release medium. The higher the amount of PLGA applied, the more acid becomes the medium. This phenomenon may have some consequences in the eye, by damaging the eye tissues. Although, the human eye aqueous humour has a high buffer capacity and it is produced at a constant rhythm which may annul this pH drop and keep the tissues intact (Figure 15).

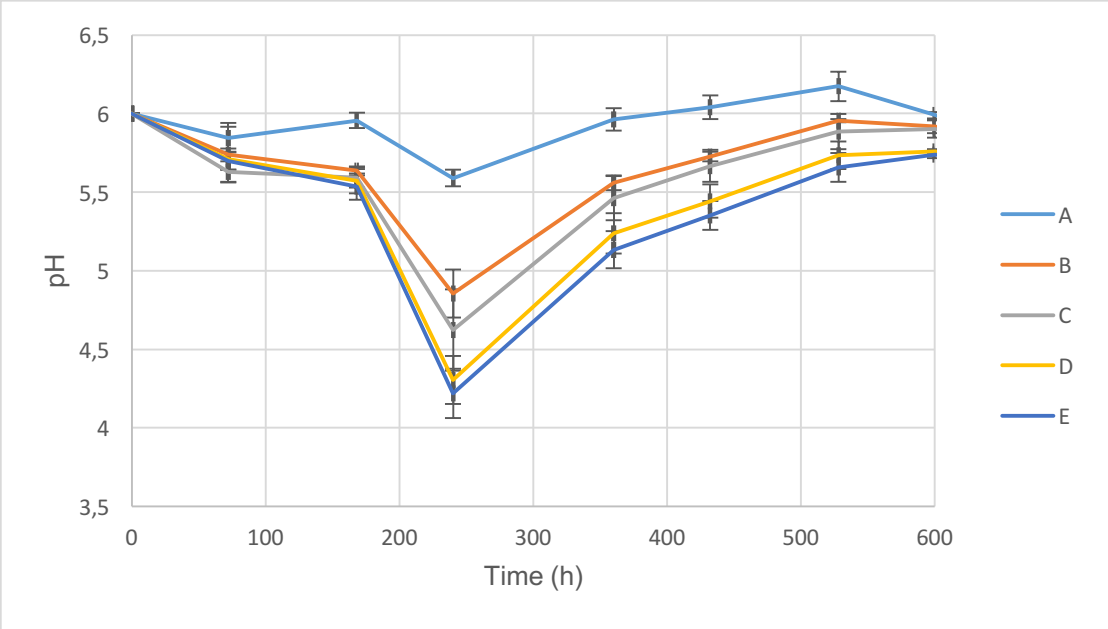

Figure 15: pH measurement of release medium from coated lenses

By increasing top coating amount of PLGA, the release was more prolonged and the initial burst release (first week) typical for PLGA formulations was reduced from 40% to 20% (Figure 16). Due to the hydrophobic nature of dexamethasone, the release was prolonged and finished after 3 months. Afterwards a similar experience was performed using NaCl 0.9% instead of the phosphate buffer as release medium and the release of the API from the lenses was faster (around 1 month). This indicates that there might be an interaction between the dexamethasone or the PLGA and the phosphate present in the artificial aqueous humour that will retard the release.

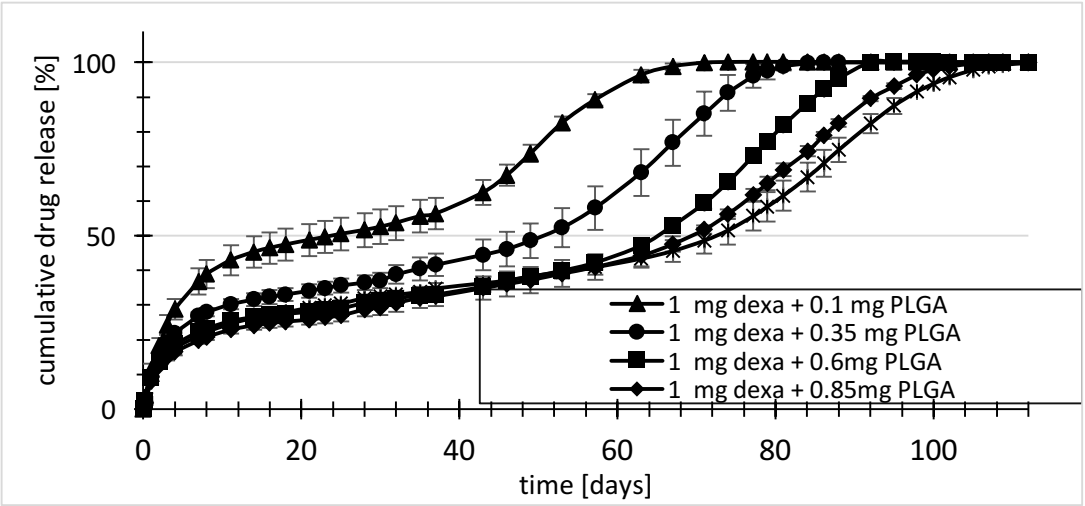

Figure 16: Cumulative release in increasing coating amount

When changing the layers of API coating, the drug loading increased or decreased without changing the release profile (Figure 17).

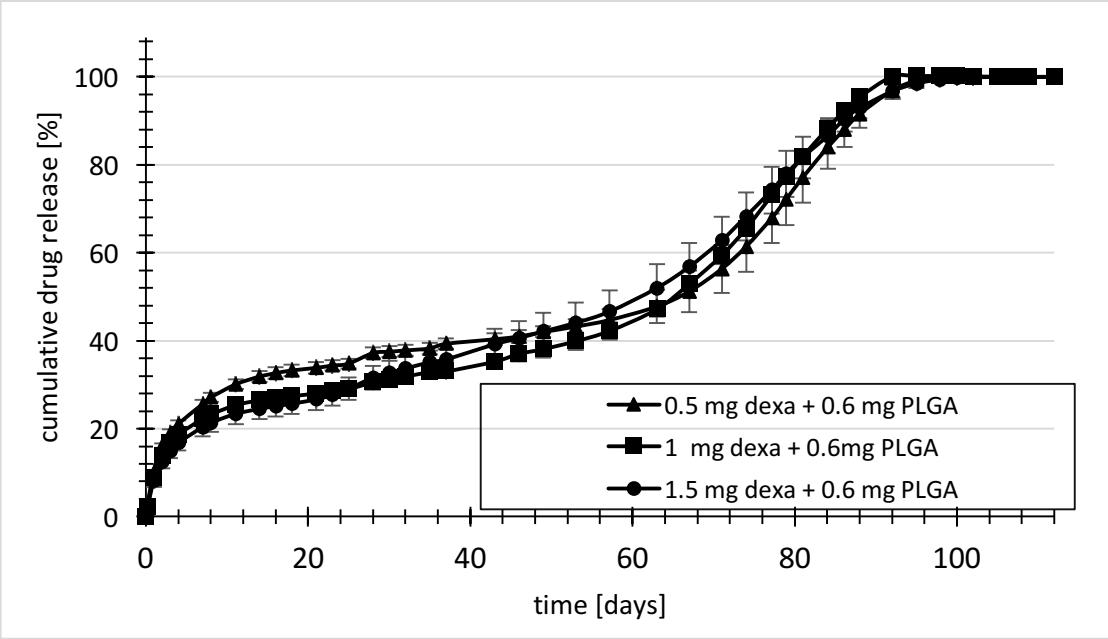

Figure 17: Cumulative release of different dexamethasone amounts

The coating on the lateral surfaces did not affect the optical properties of the lenses and the transmittance was 100% (Figure 18). However, since this technique requires a lot of precision, some of the lenses had displaced coatings which lead to adverse effects in the optical properties, especially in the transmittance (Figure 19).

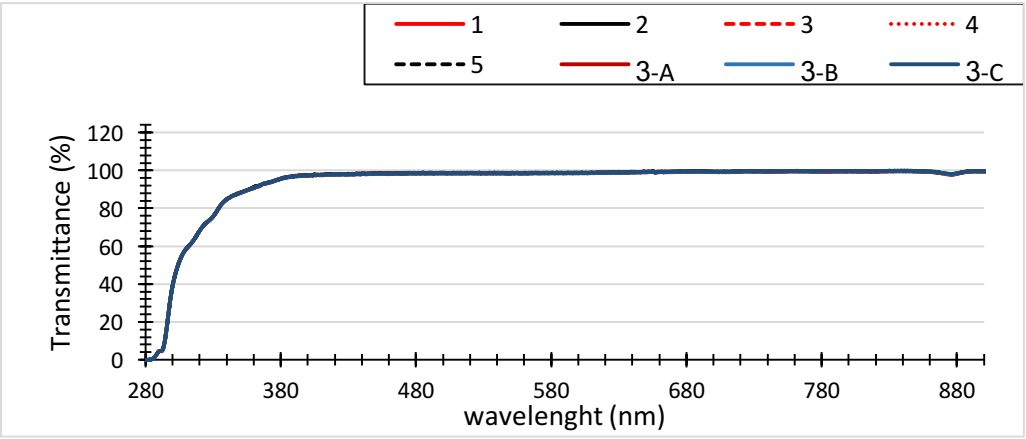

Figure 18: Transmittance at visible wavelength of coated IOLs

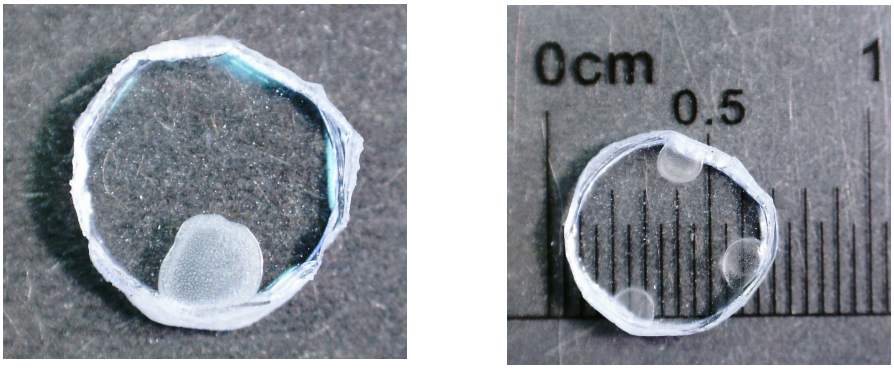

Figure 19: Displaced coatings

With this experiment, it was possible to conclude that this coating method is effective and allows an extended drug release. Besides, the addition of multiple coating layers prolonged the drug release. Consequently, the release profile might be adjusted to the desired performance, by varying the amount of biodegradable polymer used. Nonetheless, this method needs improvements in the application of the layers to avoid displaced coatings. Ideally, it would be done by an automated machine to avoid human errors.

Regarding coating technique, it is also possible to have the coating done in the haptics of the lenses. This way, the coating would not interfere with the optical properties of the lenses, if misplaced.

4 Conclusion

After the preformed experiments, it is feasible to conclude that both techniques are valid to load intraocular lenses and by changing some variables, it is possible to adjust the drug loading and release profile.

Acrylic hydrophilic intraocular lenses can be loaded by soaking technique. By optimizing the variable factors, like the formulation itself, the soaking time and the soaking medium, and perhaps some others like the soaking temperature and soaking pH, it is possible to obtain better results. For these lenses to be a good choice in the market, some practical issues need to be overcome; these lenses must be inserted in the eye in a wet state. But since this can lead to stability issues and the use of preservatives in ophthalmic formulations can be dangerous, ideally the loaded lenses would have to be delivered to the surgeon in a dry state. Then, a re-hydration step would take place in the operations room prior to the surgery takes place. Thus, to achieve this goal, it would be necessary the presence of optimal conditions that would allow the re-hydration step to be done in a short period of time, for example, 20 minutes. This re-hydration would have to be done in a very small amount of water or in a drug solution, so the loaded lenses would release the minimum amount of API. Other option consists in having a storage system with two compartments where the lens is stored in a dry state above the drug solution and when it is known when the surgery will take place, the lens would be pressed to fall into the solution and it would be soaked already in the hospital/clinic. This method would have to be improved to get a functional system and a suitable soaked amount.

Acrylic hydrophobic lenses can be loaded by coating technique. By changing the layers of API or the layers of the biodegradable polymer, it is possible to vary the amount released and the releasing profile, as it was seen before. These lenses need to be bended in order to be inserted in the eye. This can lead to a detachment of the layers; thus, it is necessary to find a surgical technique which will not damage the lens and the coating itself. Other alternatives like coating the haptics or coating the external circumference of the lenses are also to be explored and studied.

Alternative methods are being studied to load intraocular lenses, such as impregnation with supercritical fluids which allows the impregnation of API without using organic solvents (8); the use of API nanoparticles dispersed in polymers is also being studied, targeting ocular lenses (10); Cyclodextrins and liposomes have proved to be good systems to achieve a prolonged release of API (10). These methods can

be benefic to overcome some of the technical problems from the soaking and coating methods, although each one of them also has its hurdles.

Although there is not any formulation available on the market yet, these methods can be a step forward in personalized medicine. Taking into consideration the patient´s characteristics, its pathology and medical history, it is possible to adequate the therapeutics. The API and its dose can be changed according to the patient's needs. Further studies and improvements need to be done, but these innovative drug-loaded intraocular lenses are on the path to improve the quality of life of cataract surgery patients´, decreasing complications like PCO and inflammation. Also, these IOLs are especially more benefic for elderly patients who have a higher difficulty in applying the eye-drops and do not have assistance at home. With these lenses, the need to apply eye-drops is reduced, which will increase the patients´ compliance and hopefully, will reduce the incidence of second cataracts. Thus, achieving a better rate of success in cataract surgeries.

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