Dissertação para obtenção do Grau de Mestre em
Bioengenharia e Nanossistemas

Use of a surfactant (CAC) for controlling the release of drugs from hydrogels for contact lenses

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December, 2014

Abstract

The use of therapeutic contact lenses as potential systems for controlled drug release has been subject of several studies, since these devices may significantly increase the residence time of the drugs in the eye, relatively to the conventional eyedrops. However, it is not easy to maintain the drug release during the time required for an efficient treatment. Several strategies have been attempted to improve the performance of these devices, namely the incorporation of surfactants. The main goal of this work is to study the effect of the cationic surfactant cetalkonium chloride (CAC) on the release of specific drugs for ophthalmological therapy, from two hydrogels for contact lenses: one based on poly(2-hydroxyethyl methacrylate) (HEMA/PVP) and another from silicon (TRIS/NVP/HEMA). It was evaluated the effect of CAC incorporation on transmittance, ionic permeability and wettability of the hydrogels. It was concluded that CAC does not affect their transparency, but provides benefits on the wettability and ionic permeability, since it leads to the increase of both. Then, it was studied the release of two anionic anti-inflammatories (Diclofenac and Ketorolac) and a zwitterionic antibiotic (Levofloxacin), It was found that it is possible to increase the drug loading and subsequently the amount of drug released, by increasing the amount of CAC loaded in the hydrogels, as well as, in some cases, to extend the drug release duration. The Raman analysis of the drug loaded hydrogels confirmed that CAC incorporation provides a significant increase in the amount of drugs incorporated on the hydrogels. The results obtained show that the CAC incorporation is a promising method to increase the efficiency of therapeutic contact lenses.

Key-words: *Contact lenses, pHEMA based hydrogels, silicon based hydrogels, controlled drug release, surfactants*

1. Introduction

The human eye is responsible for one of the most importante sense for humans, the vision. The ocular surface is a delicate structure, vulnerable to environmental aggressions, due to its function and anatomical location [1]. External agents, like microorganisms, may produce damages that lead to different symptoms, e.g. ocular irritation, reduction of visual function and chronic changes in the tissues, including risk of infection and chronic inflammations unable to respond to treatments [2].

The pharmacological approach to treat inflammations and infections caused by microorganisms involves the administration of anti-inflammatories and antibiotics. The topical administration of the drugs is usually the most used route, since it provides higher concentrations of the drug at desired site of action when compared to oral administration [3]. This administration form is easy to use and well accepted by patients. However, it requires frequent applications, leads to significant drug losses (> 95%) and may cause undesirable side effects, due to the rapid drug absorption into the bloodstream.

The development of more efficient drug delivery systems has been subject of an increasing interest in recent years and is considered an important advance in ophthalmic therapy. Among the various possibilities studied, contact lenses have received special attention due to its high degree of comfort, biocompatibility and prolonged contact with the eye. Hydrogels are the material of choice used to manufacture contact lenses. They are polymeric materials with a high capacity to absorb water.

Typically, hydrogels are able to absorb between 40% -60% of their weight in water [4].

There are several factors that influence the swelling capacity of the hydrogels. For example, the chemical composition of the polymer network in particular the type and number of hydrophilic groups, or the crosslink density [5, 6]. The swelling ratio influences the optical and mechanical properties of the hydrogels, as well as the coefficients of diffusion and partition of solutes [7, 8].

Nowadays, different hydrogels are used in the manufacture of soft contact lenses. The traditional lenses are constituted by HEMA (2-hydroxyethyl methacrylate) based hydrogels and present excellent mechanical properties and optical clarity [9, 10]. The new generation of lenses are silicon based and combine unique properties of conventional hydrogel with the properties of silicon, namely, the high oxygen permeability [10, 11, 12].

Contact lenses may be used as carriers for controlled drug release.By being placed directly on the eye, they will increase the drugs residence time and its absorption by the target tissue [13, 14, 15]. In fact, these devices have a residence time in the cornea raging from about 12 hours to 30 days (depending on whether they are daily or monthly) and therefore have the potential to increase the bioavailability of the drug in the eye, relatively to that observed with traditional eye drops (only 1-5%) [16, 17, 18].

Several alternatives have been investigated in order to achieve the ideal drug release profiles from contact lenses. Some of them are based on nanotechnologies, such as the incorporation/adsorption of nanoparticles, nanocapsules or liposomes [15]. Others have focused on the incorporation or dispersion of specific molecules, like surfactants that may interact with the drugs and/or with the polymeric matrix and affect the drug release.

In this work, the controlled release of two anionic drugs (diclofenac and ketorolac) and a zwitterionic drug (levofloxacin) from HEMA based hydrogels (HEMA/PVP) and silicone based hydrogels (TRIS/NVP/HEMA), containing a cationic surfactant, cetalkonium chloride (CAC), will be studied.

Diclofenac sodium and ketorolac tromethamine belong to class of non -steroidal anti-inflammatory drugs (NSAIDs) and are mainly used to treat inflammation and control pain in the postoperative period.

Levofloxacin is an antibiotic belonging to the family of fluoroquinolones and shows a lethal effect on certain types of bacteria that cause ocular infections.

Due to the low toxicity of CAC it can be used in biomedical applications, in particular in contact lenses, to control the release of drugs [19, 20]. Previous studies showed that the incorporation of this and other surfactants, like Brij, in hydrogels for contact lenses, besides promoting the controlled release of drugs, improves the wettability of the hydrogels and reduces the protein binding [21-25]. This work aims to contribute to a better understanding about drugs transport through hydrogels containing surfactants and to optimize the drug release profiles to get efficient therapeutic contact lenses.

2. EXPERIMENTAL

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate, (EGDMA), 2,20-azobis(2-methylpropionitrile), (AIBN), 3 tris(trimethylsilyloxy)silylpropyl 2-methylprop-2-enoate (TRIS), levofloxacin (LVF), phosphate saline buffer (PBS), triethylamine, cetalkonium chloride (CAC), phosphoric acid, monopotassium phosphate, diclofenac sodium (DIC), ketorolac (CET) were all purchased from Sigma-Aldrich.

Poly(vinylpyrrolidone) (PVP K30, Kollidon VR 30) was kindly provided by BASF. N-vinyl pyrrolidone (NVP) and sodium chloride were obtained from Merck.

Acetonitrile and metanol were purchased from Fisher Scientific.

A Millipore Milli-Q water purification system was used to prepare distilled and deionized (DD) water.

2.2. Hydrogels preparation

To prepare HEMA/PVP based hydrogels (98/2, w/w), an appropriate amount of the crosslinker EGDMA was dissolved in HEMA (hydrophilic monomer) to obtain a concentration of 80 mM. Then, the mixture was degassed by ultra-sounds (5 min) and bubbled with a gentle stream of nitrogen (15 min) before the addition of AIBN (initiator) to a final concentration of 10 mM, and PVP (hydrophilic additive) to a final concentration of 0.02 g/mL. The solution was magnetically stirred for about 2 h to obtain complete dissolution of PVP.

In the case of TRIS/NVP/HEMA hydrogel TRIS (silicone monomer), NVP (hydrophilic additive), HEMA and EGDMA were added to the mixture with concentration of 0.94M, 3.58M, 1.53M, and 30mM, respectively. The mixture was then degassed by ultra-sounds (5 min) and bubbled with a gente stream of nitrogen (15 min) before the final addition of AIBN to obtain a concentration of 15 mM. The final mixture was magnetically stirred for about 10 min, to obtain a homogeneous solution.

Both mixtures were injected into a mould consisting of two silanized glass plates separated by a spacer of polyurethane or teflon. The glass plates

were silanized in order to facilitate the hydrogel removal from the mould. Briefly, the glasses were incubated in a 2% solution of dimethyldichlorosilane in carbon tetrachloride for 1 h and then rinsed with dichloromethane and dried with nitrogen.

In the case of HEMA/PVP, the polymerization reaction was performed at 50°C for 14 h, followed by 24 h at 70°C, while in the case of TRIS/NVP/HEMA hydrogel it occurred at 60°C for 24 h. The obtained hydrogel sheets were washed over 5 days, with DD water renewed three times a day, to remove unreacted monomers and to facilitate the cutting of the samples used in the study. The hydrated samples (thickness 0.35–0.40 mm) were cut with 1cm of diameter and dried in an oven at 35°C overnight.

2.3. CAC incorporation

First, a phosphate buffer solution (0.01 M PBS, pH 7.4.) was prepared with DD water. The cationic surfactant (CAC) was dissolved in PBS to get a 0,1% solution in CAC.

To incorporate CAC in the dry hydrogels were placed separately in tubes and immersed in 3,6mL or 7,2mL of CAC solution for different periods till 14 days, at 35 °C, to prevent precipitation, and with stirring (180 rpm), to ensure the homogeneity of the solution during the loading process. After loading, the lenses were dried in oven at 36 °C for 16 h.

The dry lenses were weighted before (Hydrogel $_{\text{dry weight bl}}$) and after (Hydrogel $_{\text{dry weight al}}$) the loading in order to estimate the loaded percentage of CAC:

$$\%CAC_{loading} = \left(\frac{Hydrogel_{dry\ weight\ al} - Hydrogel_{dry\ weight\ bl}}{Hydrogel_{dry\ weight\ bl}}\right) \times 100 \quad (1)$$

The lenses were loaded in triplicate with 5, 8, 10, 12, 15, 20 and 25% of CAC. After loading, the samples were dried in an oven at 36°C for 16h.

2.4. Hydrogels characterization

- ***Transmittance***

Transmittance of visible light (wavelength range from 400 to 700 nm) through the swollen lenses was measured using a UV–vis Beckmam DU-70 spectrophotometer.Samples were mounted on the lateral side of the outer surface of a quartz cuvette and placed in the spectrophotometer. The tests were done in triplicate before and after CAC loading (20% CAC).

- ***Wettability***

The wettability of the hydrated hydrogels was determined through the measurement of contact angles by the captive bubble method. Air bubbles were produced underneath the substrates immersed in PBS using a micrometric syringe. Several bubble images were acquired during 30 s using a video camera (JAI CV-A50) attached to a microscope (Wild M3Z) which is connected to a frame grabber (Data Translation DT3155). The image analysis was performed using the ADSA-P software (Axisymmetric Drop Shape Analysis Profile). The measurements were done at ambient temperature in triplicate, before and after CAC loading (20% CAC).

- **Ionic permeability**

The ionic permeability of the hydrogels was measured using a home-made PMMA horizontal diffusion cell. The fully hydrated hydrogel was mounted in the cell, and 24 mL of NaCl solution (130 mM) and 32 mL of DD water were placed into the donor and the receiver compartments, respectively.

The conductivity of the fluid in the receiving chamber was determined as a function of time, using a conductivitymeter (Cond 340i/ SET, WTW). The conductivity data (in μS cm^{-1}) were converted into NaCl concentration (in mg/mL) through a calibration curve previously obtained with NaClsolutions.

The rate of ion transport (F) corresponds to the slope of the linear regression applied to the concentration versus time data. Solving the diffusion equation under the pseudo-steady state conditions it is possible to determine the ionic permeability (also referred as the ionoflux diffusion coefficient, D_{ion}):

$$\frac{F \times V}{A} = D_{ion} \frac{dC}{dx} \quad (2)$$

where V is the volume of the receiver solution, A is the área of the hydrogel sample and dC/dx represents the initial NaCl concentration gradient across the hydrogel.

The experiment was performed at 36°C in triplicate before and after CAC loading (20% CAC).

2.5. Drug loading and drug release experiments

- ***Drug loading***

The dry hydrogel samples (without CAC and containing different percentages of CAC) were loaded with the drugs (diclofenac, cetorolac and levofloxacin) by soaking in the drug solution (prepared with PBS) with a concentration of 1 mg/mL, for 38h, at ambient temperature, in the dark.

The volume of loading drug solution was calculated by using the following formula:

$$V_{loading\ drug} = \frac{\left(\frac{(\%\ CAC\ \times 140)}{8.5} \times W_{dry\ lens}\right)}{1000} \quad (3)$$

The loaded samples were rinsed with PBS and dried with absorbent paper.

- ***Sink coditions***

In vitro drug release experiments were carried out for 200 h to determine drug release profiles. The drug loaded samples were immersed in 4mL of PBS solution in closed vessels, at 35°C, under stirring (180 rpm). At predetermined time intervals, 800µl aliquots of the supernatant were collected and replaced by the same volume of fresh PBS solution.

- ***Release in physiological conditions***

A microfluidic cell was used to approach the physiological conditions found in the eye (tear volume 45µL and PBS flow rate 3µL/min).

The experiments were carried out at 35°C in triplicate. Aliquots were collected every hour.

- ***Drug quantification***

The concentration of diclofenac, cetorolac and levofloxacin in the supernatant was determined using a high performance liquid chromatograph (HPLC) with a Jasco UV–vis detector and a C-18 column Nova-Pak Watters, at wavelengths of 276, 323 and 290 nm, respectively.

The mobile phases consisting of phosphoric acid, acetonitrile and methanol (40/48/12 in volume) for diclofenac, methanol and phosphate buffer pH 3 (55/45) for cetorolac and water, acetonitrile, phosphoric acid and triethylamine (86/14/0.6/0.3 in volume) for levofloxacin,were introduced into the column at a flow rate of 1 mL/min and a pressure of 14 MPa.

2.6. Raman spectroscopic analyses

Raman spectra were collected in the range 200–1800 cm-1 using a LabRAM HR Evolution Confocal Microscope (Horiba Scientific) with 633 nm excitation. The light was focused with a 100×, NA=0.9, WD= 0.21 mm objective. The laser power on the sample without any neutral density filters was ≈ 10mW.

The Raman signal was detected with a Peltier-cooled (-70°C) Horiba Synapse CCD detector with 1024 × 256 pixels. Spectra were acquired using 5 s of signal collection time and 10 accumulations. Labspec 6 software (Horiba Scientific) was used to analyze all spectra through background subtraction and peak fitting.

3. RESULTS AND DISCUSSION

- ***Hydrogels characterization***

Two types of hydrogels were studied: HEMA/PVP, a conventional hydrogel, and TRIS/NVP/HEMA, a silicone hydrogel. Besides the presence of the silicone monomer (TRIS), a significant difference between the compositions of both hydrogels concerns the amount of cross-linking agent which is about three times more concentrated in HEMA/PVP. In general, increasing the cross-link density within a hydrogel network improves its mechanical properties, but decreases the water-induced swelling capacity of the hydrogel [43].

The values of transmittance obtained for HEMA/PVP and TRIS/NVP/HEMA with 0 and 20% of CAC are represented in Figure 1.

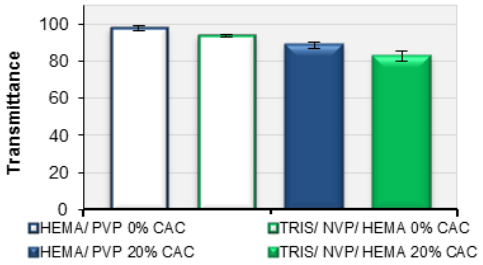

Figure 1 - Average transmittances (λ=400-700 nm) of HEMA/PVP and TRIS/NVP/HEMA with 0 and and 20% CAC, hydrated in PBS, obtained at ambient temperature (25 ° C).

The incorporation of a high proportion of surfactant (20%) leads to a decrease in transparency of the hydrogels, since at ambient temperature the CAC may precipitate and cause some opacity.

This is does not happen at physiological temperature (36 °C), but due to equipment limitations it was not possible to measure the transmittance at this temperature.

However, the results suggest that the lenses loaded with CAC (with this % or lower) must have transmittances within the ranges reported in the literature for contact lenses (> 85%) [26, 27, 28].

The average water contact angles obtained for HEMA/PVP and TRIS/NVP/HEMA with 0 and 20% CAChydrated in PBS are shown in Figure 2.

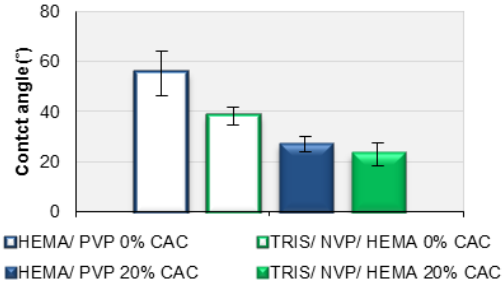

Figure 2 - Average contact angles of HEMA/PVP and TRIS/NVP/HEMA with 0 and 20% CAC, hydrated in PBS, obtained by the captive bubble method.

For incorporation of CAC in HEMA/PVP leads to a decrease in the contact angle from 55º to 27º. In TRIS/NVP/HEMACAC also induces an improvement in wettability, since hydrogels without CAC have an average contact angle of 38º that decreases to 23° in the presence of CAC.

This behavior may be explained by the reorientation of the polar groups of the surfactant to outside part of the surface, promoting the interaction with water [29,30].

Figure 3 presents the diffusion coefficients obtained for the studied hydrogels with 0 and and CAC.

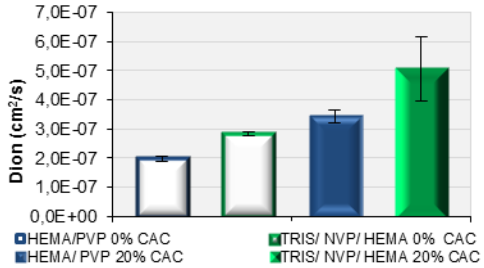

Figure 3 - Average diffusion coefficients of the hydrogel HEMA/PVP and TRIS/NVP/HEMA with 0 and 20% CAC.

The ionic permeability of TRIS/ NVP/HEMA is higher than HEMA/PVP. In both cases, the addition of CAC increases considerably the ionic permeability of the hydrogels. The values of the diffusion coefficients are in all cases above the minimum acceptable to ensure the mobility and comfort suitable for contact lenses. i.e. 1,067 x 10^{-9} cm^2s^{-1} [4,31].

- ***Drug release profiles***

Diclofenac

Absolute and percentual cumulative release profiles for diclofenac release from TRIS/NVP/HEMA and HEMA/PVP, in function of CAC percentage, are present in Figure 4 and 5, respectively.

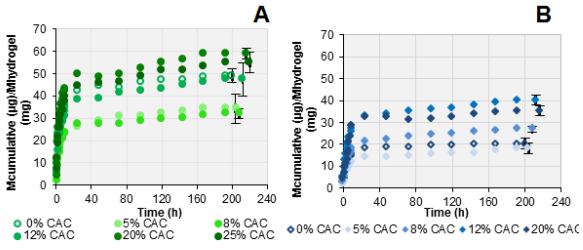

Figure 4 – Absolute cumulative release profiles of diclofenac from hydrogels TRIS/NVP/HEMA (A) and HEMA/PVP (B), for different percentages of CAC incorporated.

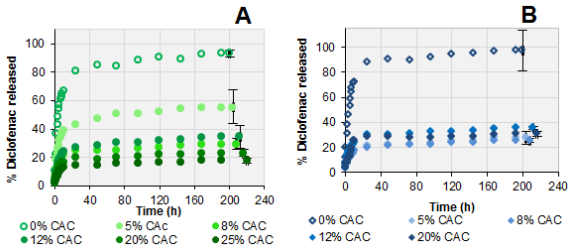

Figure 5 – Percentual release profiles of diclofenac from the hydrogel TRIS/NVP/HEMA (A) and HEMA/PVP (B), for different percentages of CAC incorporated.

For all the release profiles it is found that for the first 24 h, there was a controlled release of diclofenac, and from here the rate of diclofenac release was much smaller, sometimes neglectable, until 200 h. In a general way, the higher the percentage of incorporated CAC, the higher is the absolute amount of diclofenac releasedDepletion data allowed to calculate the percentage of drug released. It is observed that for higher percentages of CAC, lower percentages of diclofenac are released. This suggests that part of the drug was retained into the matrix due to the strong electrostatic interactions between the drug and the surfactant.

The partition coefficients (K) for diclofenac in both hydrogels are present in figure 6.

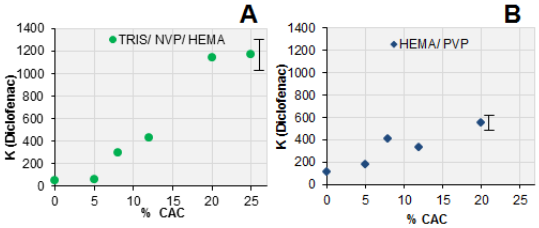

Figure 6- Partition coefficient (K) of diclofenac for hydrogels TRIS / NVP / HEMA (A) and HEMA/PVP (B) in function of CAC percentage.

The partition coefficients increase with the percentage of incorporated CAC, indicating that higher amounts of CAC lead to higher diclofenac contents. However they achieve significantly higher values for TRIS/NVP/HEMA (1140 against 550 in HEMA/PVP). This suggests a higher tendency for loading the drug for silicone hydrogel.

According to *Bengani et al* [20], the way in which the partition coefficient varies with the percentage of CAC can give indications about the aggregation state of the surfactant in the matrix: a linear fitting indicates no aggregation, while exponential fitting suggests the formation of aggregates. In this case, the fitting results were not conclusive.

Diclofenac release profiles from TRIS/NVP/HEMA e HEMA/ PVP without CAC were obtained using a microfluidic cell which approaches the physiological tear flow hydrodynamics (Figure 7).

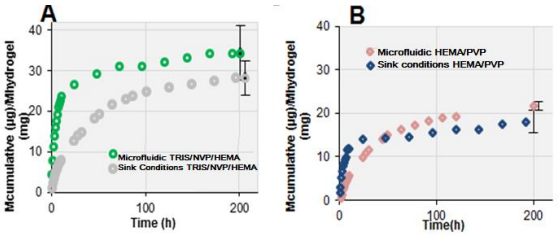

Figure 7- Comparison of diclofenac release profiles from TRIS / NVP / HEMA (A) and HEMA / PVP (B) in sink conditions and physiological flow.

In both cases it was found that with the use of microfluidic cell, the release of diclofenac extends for 8 days, while in sink conditions it is much faster. It is also verified that the drug concentration is above the MEC (50ng/mL) for 8 days, ensuring a more effective drug than the drops (not shown).

Ketorolac

Absolute and percentual cumulative release profiles for cetorolac are presented in Figures 8 and 9 for TRIS/NVP/HEMA and HEMA/PVP, respectively

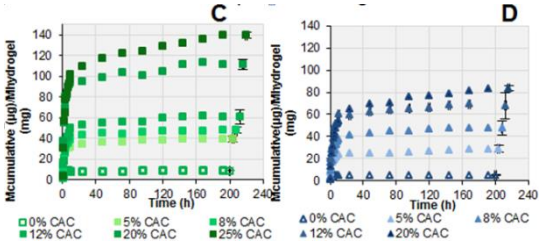

Figure 8 – Absolute cumulative release profiles of ketorolac from hydrogels TRIS/NVP/HEMA (C) and HEMA/PVP (D), for different percentages of CAC incorporated

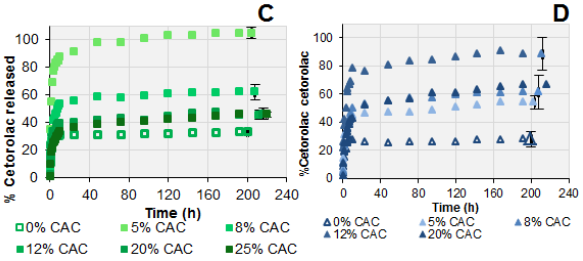

Figure 9 - Release profiles, in percentage of cetorolac from the hydrogel TRIS / NVP / HEMA (C) and HEMA/PVP (D), for different percentages CAC incorporated.

As expected, higher percentages of CAC lead to higher amounts of ketorolac released. As in the case of diclofenac, the releaseof ketorolac occurs mainly in the first 24 h. For hydrogels without CAC, the complete release of the drug occurred during the first 2 hours.

The quantities of keterolac released from the hydrogels are higher than that of diclofenac. By analyzing the percentages of ketorolac releasedfrom the TRIS/NVP/HEMA, it was observed that the higher the percentage of CAC, the lower the percentage of drug released, as it happened with diclofenac. However, for HEMA/PVP this tendency was not observed.

The partition coefficients (K) of ketorolac are presented in Figure 10.

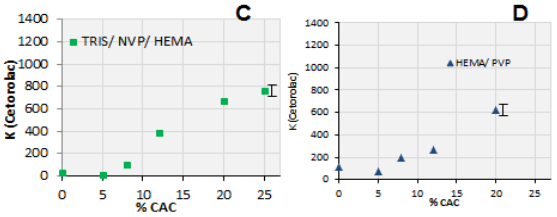

Figure 10- Partition coefficient (K) of ketorolac for hydrogels TRIS/NVP/HEMA (C) and HEMA/PVP (D) for different percentages of CAC.

It is observed an increase with the percentage of CAC in both cases, although the values for TRIS/NVP/HEMA do not achieve those estimated for diclofenac. For this hydrogel, the values ajust to an exponential function, suggesting that the surfactant molecules are directly bound to the polymeric matrix.

Ketorolac release profiles from were obtained using a microfluidic cell (Figure 11).

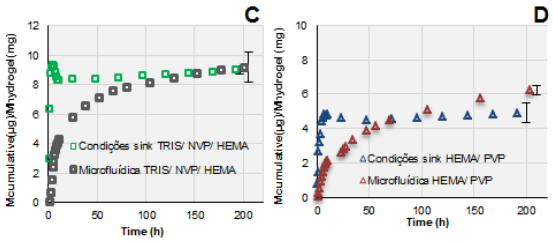

Figure 11- Comparison of ketorolac release profiles from TRIS/NVP/HEMA (C) and HEMA/PVP (D) in sink conditions and physiological flow.

The ketorolac release obtained in hydrodynamic conditions provided an extend drug release for 8 days, while in sink conditions the release is much faster. The drug concentration remains above the MEC (40ng/mL) for the 8 days.

Levofloxacin

Absolute and percentual cumulative profiles of levofloxacin released are presented in Figures 12 and 13.

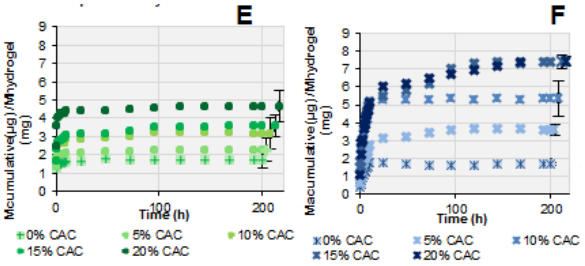

Figure 12 – Absolut cumulative release profiles of levofloxacin from hydrogels TRIS/NVP/HEMA (E) and HEMA/PVP (F), fordifferent percentages of CAC incorporated.

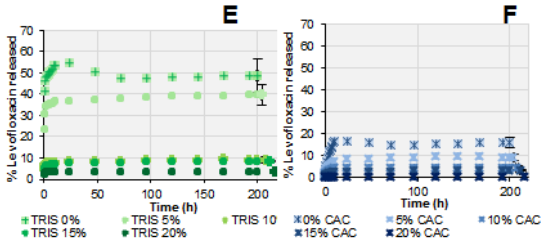

Figure 13 – Percentual release profiles of levofloxacin from the hydrogel TRIS/NVP/HEMA (E) and HEMA/PVP (F), for different percentages of CAC incorporated.

As shown, the higher the percentage of CAC incorporated, the higher is the mass of levofloxacin released. However, it is evident that the amount of levofloxacin released is much lower than of the anti-inflamatories previously referred. The release kinetics from HEMA/PVP is similar to the observed with the other drugs (most of the drug released in 24h), but is faster for TRIS/NVP/HEMA (release in less than 2h). The percentage of levofloxacin released decreases with the increase in CAC content, in agreement with what was observed in previous systems. Levofloxacin release percentages from HEMA/PVP are the lowest of all the systems studied, indicating that most of the antibiotic is retained in the polymeric matrix.

As before, also the partition coefficient (Figure 14) of levofloxacin increases with the percentage of CAC.

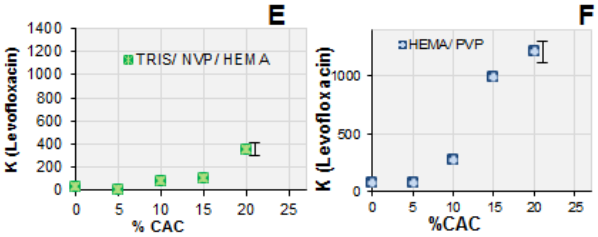

Figure 14- Partition coefficient (K) of levofloxacin for hydrogels TRIS/NVP/HEMA (E) and HEMA/PVP (F) for differents percentages of CAC.

It is notorious that K for the hydrogel HEMA/PVP reaches significantly higher values than for TRIS/NVP/HEMA, which demonstrates that this antibiotic has an higher affinity to the HEMA based hydrogel.

- ***Interaction between drugs, surfactant and hydrogels***

In order to understand the differences between the release results for each drug after CAC incorporation, Raman spectroscopy was used to investigate possible interactions between drugs, polymers and CAC. In fact, the drug transport across hydrogels depends not only on the characteristics of the polymer matrix and drug, but also on the established interactions between them, that somehow affect theloading and diffusion of the drug [43,119].

In Figure 15 are shown the Raman spectra obtained for TRIS/NVP/HEMA and HEMA/PVP in the presence and absence of diclofenac and CAC loading (20%).

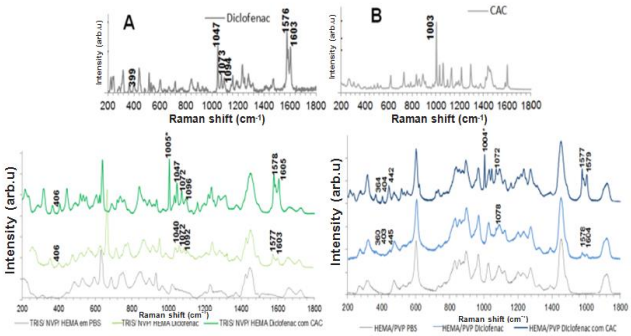

Figure 15- Raman spectra for TRIS/NVP/HEMA (bottom left) and HEMA/PVP (bottom right) hydrated in PBS, loaded with diclofenac and loaded with CAC and diclofenac; Spectrum of pure diclofenac (top, A); Spectrum of CAC (top, B); Peaks marked with * belong to CAC.

The incorporation of diclofenac into the hydrogel TRIS/NVP/HEMA gave rise to the appearance of peaks at 406, 1040, 1072, 1092, 1577 and 1603 cm^{-1}. These peaks are common to diclofenac (Figure 15 A) and hence indicate the presence of the drug in the hydrogel matrix. The spectrum of the hydrogel with diclofenac and CAC exhibits an additional peak at 1005 cm^{-1}, corresponding to the most intense peak of CAC (Figure 15 B). Note that, although these peaks present small deviations relatively to the pure drug and the surfactant, specific interactions between the three compounds could not be identified [32].

In the case of hydrogel HEMA/PVP, the presence of diclofenac gives rise to the appearance of peaks at 360, 403, 445, 1078, 1578 and 1604 cm^{-1}, characteristic of the drug. In the simultaneous presence of drug and surfactant, these peaks become still more intense and a peak characteristic CAC appears at 1004 cm^{-1}. This increase in intensity is common for both HEMA/PVP and TRIS/NVP/HEMA hydrogels and indicates that CAC incorporation increases the amount of drug present within the hydrogels matrix. Qualitatively we observed that the intensity of diclofenac peaks is higher in hydrogels TRIS/NVP/HEMA, corroborating the results of depletion (not shown).

Figure 16 shows the equivalent Raman spectra for cetorolac.

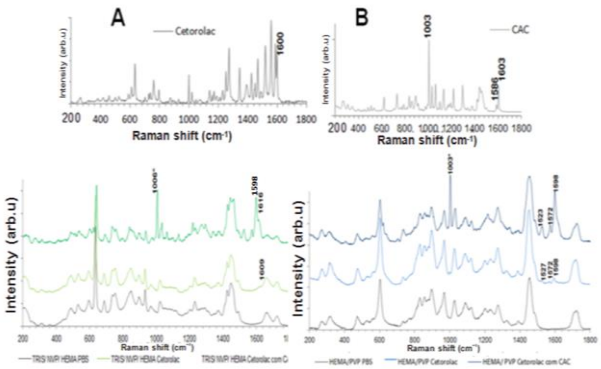

Figure 16- Raman spectra for TRIS/NVP/HEMA (bottom left) and HEMA/PVP (bottom right) hydrated in PBS, loaded with ketorolac and loaded with CAC and ketorolac ; Spectrum of pure ketorolac (top, A); Spectrum of CAC (top, B); Peaks marked with * belong to CAC.

Comparison of the spectra of TRIS/ NVP/HEMA with and without keterolac only allows to detect differences in the region of 1609 cm^{-1}: a small peak appears in the spectrum of the loaded hydrogel at this wave number, which can be attributed to the drug. The loading with CAC and ketorolac induces the appearance of an intense peak at 1006 cm^{-1} and several other significantly smaller peaks between 1031 and 1575 cm^{-1}, which are common to the CAC and the drug. A very intense peak appears at 1598 cm^{-1}, which arises from overlap of the CAC and keterolac peaks at this wave number. The results suggest that in the absence of CAC, the amount of loaded drug should be minimal. However, with 20% CAC in the matrix, it is possible to observe significant changes in the spectrum which suggest the presence of a larger amount of drug. The overlapping of the CAC and ketorolac peaks in the region of 1000 to 1600 cm^{-1} does not allow distinguishing between CAC contribution and the ceterolac and therefore, it is not possible to infer, even qualitatively, on the relative amount of drug that is present inside the hydrogel matrix.

With respect to HEMA/PVP hydrogel, it was observed that the incorporation of keterolac in the absence of CAC generates three small peaks characteristic of the drug, at 1527, 1572 and 1598 cm^{-1}, which confirm its presence. When CAC is pre-incorporated, the peaks intensity increases, possibly due to the higher amount of drug incorporated and the overlap with some peaks of CAC. An intense peak appears at 1003 cm^{-1} which could be attributed mainly to the surfactant. Thus, it is concluded that the presence of CAC should promote the loading of ketorolac in the HEMA/PVP matrix.

Figure 17 shows the Raman spectra obtained in the study with levofloxacin.

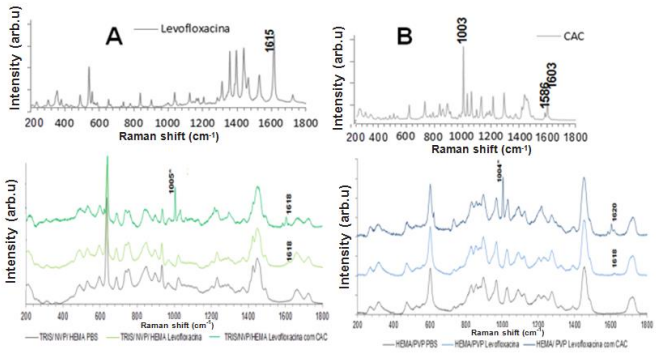

Figure 17- Raman spectra for TRIS/NVP/HEMA (bottom left) and HEMA/PVP (bottom right): hydrated in PBS, loaded with levofloxacin and loaded with CAC and levofloxacin; Spectrum of pure levofloxacin (top, A); Spectrum of CAC (top, B); Peaks marked with * belong to CAC.

For levofloxacin, the most important fact to note is the presence of the most intense peak of CAC in both hydrogels loaded with drug and CAC and a characteristic levofloxacin peak at ≈1618 cm^{-1}, which is more intense in the presence of CAC. Indeed, in both cases, it appears that the incorporation of CAC slightly increases the intensity of the characteristic peak of levofloxacin. Qualitatively, we observed higher intensity for HEMA/PVP which corroborates the results of depletion (not shown).

4. Conclusions

In this study we investigated the effect of cationic surfactant incorporation (cetalkonium chloride, CAC) in hydrogels for therapeutic contact lens to control the release of drugs.

To this end, two hydrogels were prepared, one based on poly (2-hydroxyethyl methacrylate) (HEMA/PVP) and another of silicone (TRIS/NVP/HEMA).CAC was incorporated in different amounts in these materials by immersion in solutions of the surfactant.

The effect of CAC incorporation in materials properties crucial for their performance incontact lenses, particularly in transmittance, ion permeability and wettability, was evaluated. With respect to transparency, it was found that, although at room temperature, high CAC percentages may origin the surfactant precipitatation in the polymeric matrix and cause some opacity, at physiological temperature (36° C) this does not happens:transmittance values are within those generally reported in the literature for contact lenses. With regard to wettability, CAC incorporation increases the hydrophilicity, because the surfactant present on the surface reorients the polar groups outwards, promoting interaction with water. The ion permeability of the two hydrogels increases considerably with the addition of CAC (the diffusion coefficient increases), specially for TRIS/NVP/HEMA which is less crosslinked and presents an higher amount of water in the hydrated

state. Both the increase in wettability and in the ion permeability are benefic to enhance the comfort provided by the lenses materials.

As expected, CAC has a strong link with the polymeric matrix. Thus, no signs of its release were detected.

Two anionic anti-inflammatories (diclofenac, and ketorolac) and a zwitterionic antibiotic (levofloxacin) were incorporated into the studied hydrogels, by immersion in solutions of the drug. In all cases, it was found that the higher the percentage of CAC incorporated into the hydrogels, the greater the amount of drug loaded.

The release of drugs was studied in sink conditions. It was observed that in all cases, the increase in the CAC content in the matrix leads to an increase of absolute amount of drug released. Comparing the results obtained with the different drugs, it is evident that the released amount of levofloxacin is substantially lower than the anti-inflammatories, both for HEMA/PVP and TRIS/NVP/HEMA. However, it is interesting to note, from the depletion data, that, despite the amounts of drug loaded into the hydrogels without CAC are significantly lower in the case of levofloxacin, for hydrogels loaded with e.g. 20% CAC (the same content of those used in Raman studies), the quantities of loaded levofloxacin are of the same order of magnitude as observed for the anti-inflammatories. This suggests a preferential interaction of levofloxacin with CAC, which may not necessarily be of electrostatic nature (since the CAC is cationic and levofloxacin is a zwitterionic antibiotic).

The percentual drug release profiles demonstrate that, since plateau values of 0.1% to 3.5% were found for the release of levofloxacin from HEMA/PVP and TRIS/NVP/HEMA, respectively, loaded with 20% CAC, and percentages between 23 and 66% were observed for the release of the anti-inflammatories from hydrogels with the same CAC content. It is still interesting to mention the fact that, in general, for all drugs,the greater the percentage of CAC incorporated, the lower the percentage of drug released (ceterolac in HEMA/PVP is an exception, since it is leads to an opposite behaviour to some extent, and diclofenac inHEMA/PVP also, since in this case the releaseis little affected by the CAC content). This shows that in all cases the drug form strong interactions with CAC, which hinder their diffusion into the hydrogel and prevents the release of a significant amount of drug.

Surprisingly, it was expected that the strongest interactions were with anti-inflammatories, because these are anionic and CAC is cationic, and can therefore establish electrostatic interactions, but as stated above, other interactions (between drug and surfactant and/or drug and polymer matrix) should determine the greater retention of levofloxacin.

Note that the interaction of the drug with the polymer matrix (without CAC) also varies from case to case: while for diclofenac interaction is weak either with HEMA/PVP or with TRIS/NVP/HEMA (the release percentages without CAC are greater than 93%) with other drugs should establish more significant interactions (since the cumulative release percentages vary between 16 and 48%).

It is also interesting to compare the amounts of each drug loaded and released from each type of hydrogel (regardless of the presence of CAC). Both for diclofenac and ketorolac, larger values are , in general, observed for drug loading and cumulative mass released in TRIS/NVP/HEMA. This event may be related to the fact that both drugs are hydrophilic. Higher diffusion is expected in hydrogels with higher water content and more hydrophilic surfaces (in the hydrated state), as is the case of the silicone hydrogel. In the case of levofloxacin, it was found, as expected, the opposite, since it will have a higher affinity for lipophilic hydrogels with lower water content (HEMA/PVP). It was still found that the hydrogels HEMA/PVP have more control in the release of levofloxacin, probably due to the large crosslinking density of this.

Regarding the drug release kinetics, it was observed that for all CAC percentages the drug release takes place mainly in the first 24h. The presence of CAC led, in certain cases, to a slight increase in the release time of the drugs.

The drug partition coefficients in the hydrogels were calculated for the different contents of CAC. As expected, it was observed a significant increase with the amount of CAC incorporated. The magnitude of the values is in agreement with that found by other authors and indicates that at equilibrium, the amount of drug that is found inside of the hydrogel is substantially greater than it would be in the same volume of solution [80]. The adjustment of the variation of the partition coefficients with the percentage of the CAC to linear or exponential functions only allowed to infer about the surfactant state of aggregation in the case of TRIS/NVP/HEMA with diclofenac and TRIS/NVP/HEMA with ketorolac. In the other cases, the proximity of the correlation coefficients obtained for the fittings did not allow to get conclusions.

In order to help to understand the differences in the release of drugs in the various systems studied, Raman spectroscopyanalysis was done. The results did not allow to identify specific interactions between the hydrogel, the surfactant and the drugs, probably due to the overlapping peaks of the drug and the surfactant. However, it was confirmed that the incorporation of CAC significantly increases the amount of drug present in the polymeric matrices.

Finally, release tests of diclofenac and ketorolac from the hydrogels HEMA/PVP and TRIS/NVP/HEMA (without CAC) were conducted using a microfluidic cell which approaches the hydrodynamic conditions of the eye. It was found that under these conditions, the release of drugs is prolonged for about 8 days, while in sink conditions release is very fast, not exceeding 1 day.

It was concluded that, as expected, CAC incorporation influences the loading of the drug and subsequent release, which is reflected not only in the increase of the drug mass released, but also, in certain cases, by increasing the release time. There were also improvements of essential properties for the *in vivo* good performance of contact lenses, such as wettability and ion permeability, which influence into a large extent the eye health and comfort of the user. In summary, CAC incorporation into the studied systems is a promising technique for achieving an increase in the bioavailability of drugs, by improving the effectiveness of ocular treatments with therapeutic lenses.

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