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Research paper

# Design of poly(ethylene glycol)-tethered copolymers as novel mucoadhesive drug delivery systems

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## Abstract

We developed novel acrylic-based polymers that can be used as mucoadhesive delivery systems. Poly(acrylic acid) hydrogels were modified by grafting adhesion promoter chains such as poly(ethylene glycol) (PEG) onto their back-bone chains, thus promoting the adhesive process by interpenetration. The copolymers synthesized were designated as P(AA-g-EG). Hydrogels were synthesized using PEG of two different molecular weights, 1000 and 2000, and with varying molar feed ratio of AA–EG (20:80, 40:60, 60:40, 80:20, 12:88, 25:75, 44:56, 67:33). The copolymers were synthesized by using free radical solution UV-polymerization. The effects of different PEG-tethered structures on mucoadhesion were studied using a tensiometric testing and the work of adhesion was calculated. Preswollen P(AA-g-EG) copolymer films composed of 40% acrylic acid (AA) and 60% ethylene glycol (EG), containing PEG 1000 tethers, exhibited the highest value for the work of mucoadhesion, $130\times10^{-3}\pm27\times10^{-3}$ mJ, that is five times higher than the formulation composed of pure PAA. Based on these results and associated molecular analysis, we conclude that the higher mucoadhesive properties of this specific copolymer were the result of the synergistic effects of both monomers. AA functional groups allowed the polymer to form multiple hydrogen bonds with the glycoproteins present in the mucus. PEG tethers possibly acted as mucoadhesive promoters, enhancing interpenetration of polymer chains into the mucus.

*Keywords:* Mucoadhesive hydrogels; Poly(acrylic acid); Poly(ethylene glycol)-tethered structures; pH-Sensitive copolymers; Drug delivery

## 1. Introduction

Mucoadhesive controlled drug delivery systems are very beneficial, since they provide a controlled drug release over time and localize the drug to a specific site of the body [1]. The prolonged residence time of the drug in the body is believed to prolong duration of action. Mucoadhesive drug delivery devices can be applied to any mucosal tissue in the body including the gastrointestinal, ocular, respiratory, buccal, nasal, rectal, urethral and vaginal path [2,3]. Oral administration of drugs is the preferred administration route for the purpose of this work. Since, the GI tract is covered by a mucus layer, localization of a mucoadhesive drug delivery system to a specific site is very beneficial.

Challenges for oral mucoadhesive systems include the harsh environment of the stomach, which, due to its low pH, results in an activation of a wide range of drugs as well as the degradation of protein drugs administrated for therapeutic purposes. On the other hand, the wash out effect in the GI tract due to the intestinal motility results in a low residence time of the drug at the site of absorption.

The use of acrylic-based polymers can be very beneficial to overcome the shortcomings of oral drug administration. Acrylic-based polymers have been extensively used for mucoadhesive applications, since they exhibit very high adhesive bond strength in contact with tissues [4–6]. Thus, they allow the localization of the drug at the site of absorption, increasing its residence time at the absorbing tissue and increasing drug bioavailability. Also, they exhibit a pH responsive behavior, which allows the release of the drug at the desirable site of the GI tract. Consequently, the drug may be released in the upper small intestine and protected from the low pH of the stomach.

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The mucoadhesive properties of these hydrogels can be improved by molecularly designing the polymeric systems. In this work, hydrophilic polymers were modified by grafting adhesive promoters such as poly(ethylene glycol) (PEG) onto their back-bone chains, thus promoting their adhesive behavior. The novel copolymers obtained are designated as P(AA-g-EG) and can be classified as complexation hydrogels [7–9]. Such copolymer networks exhibit pH-dependent swelling behavior due to the formation and dissociation of interpolymer complexes. In acidic media, the copolymer is in a collapsed state due to the hydrogen bonding between the ether group of the PEG chain and the carboxylic group of the PAA. In a basic environment, above the $pK_A$ value of AA, equal to 4.26, the pendant groups ionize and the complexes dissociate. Because of this phenomenon, the network presents large changes in its structure, permitting the adhesion of the copolymer at a specific location in the body and the release of drugs and proteins at a desirable rate [10].

### 1.1. Mucoadhesion

Mucoadhesion is the attachment of a natural or synthetic polymer to the mucosa [1]. Strong interactions between the chemical groups of the polymer carrier and the mucous lining of the tissue permit the attachment of a polymer to the mucosa. The mucus lubricates and protects the epithelial tissue from mechanical and chemical damage. Although the mucus secretion can vary depending on age, sex, part of the body, and pathological conditions, the average turnover rate in humans is approximately 6 h [11]. The main components of the mucus are glycosylated proteins named mucins. Mucins consist of a protein core with carbohydrate chains covalently attached over its length. These glycoproteins are the responsible for the gel-like properties of the mucus. Most of the mucoadhesive bonds are attained by either physical or secondary chemical bonds, the predominant interaction being hydrogen bonding [12]. Consequently, several functional groups such hydroxyl, carboxyl and other hydrogen bond-forming functional groups can contribute to mucoadhesion [13].

Several theories have been proposed in order to explain the mucoadhesive phenomenon. The mucoadhesion theories developed over the time include: the electronic theory [14], the wetting theory [15], the adsorption theory [16], the fracture theory [12,17] and the diffusion theory [18]. Taking into account all these theories, the process involved in the formation of mucoadhesive bonds can be divided in four basic steps: wetting and swelling of the polymers; interpenetration of the mucoadhesive polymer chains and entanglement of the polymer and mucin chains; interfacial interaction of functional groups; and formation of weak chemical bonds.

### 1.2. PEG and its role as a mucoadhesion promoter

Interpenetration of polymer chains at the interface is an important mucoadhesion mechanism. When the polymer gets into contact with the mucus, the concentration gradient at the interface provokes the spontaneous diffusion of the polymer chains into the mucus layer, and also the diffusion of the mucin glycoproteins into the polymer. Jabbari et al. [19] demonstrated the importance of such chain interpenetration involved in the mucoadhesion phenomenon, by a successful application of ATR-FTIR technique. Specifically they studied the chain interpenetration of PAA polymers into mucin glycoproteins.

Based on these results, we studied enhancement of interpenetration. In early studies in our laboratories, DeAscentis et al. [20] proposed the use of linear polymer chains, or adhesion promoters, when designing mucoadhesive materials, in order to enhance the adhesion of polymers onto the mucosa. An adhesion promoter can be defined as a molecule that is not mucoadhesive in itself but contributes into the overall adhesion process. Other work in our lab [1] showed that, for example, PEG chains can be used as mucoadhesion promoters.

In addition, polymer chains that are tethered or grafted onto polymeric surfaces are also able to act as mucoadhesion promoters [21,22]. Tethered chains have one of their ends covalently attached on the polymer surface and leave the other free, preventing the loss of those chains. The grafted chains are still able to diffuse from the polymer network to the mucus, enhancing interpenetration and aiding the mucoadhesion phenomenon [23,24]. Huang et al. [24] studied the use of PEG-tethered polymers as novel mucoadhesive materials, using the single-chain mean-theory [25]. Also, experimental conclusions were obtained from studies realized with PEG and mucin assembled surfaces [26]. Some of those conclusions help interpret the experimental results presented in this work. In our case, the study of PEG-tethered structures is brought to a more realistic situation where, instead of using PEG assembled surfaces [26], potential mucoadhesive drug delivery carriers containing PEG tethers are synthesized. Fig. 1 shows the chain interpenetration of PEG tethers into the mucosal layer.

The goal of this contribution was to improve acrylic-based polymers, used as carriers in the attachment of mucoadhesive drug delivery systems to the mucosa. Polymer hydrogels were modified by grafting PEG onto the backbone chains of the polymer, and consequently promoting the mucoadhesive process. We were interested in elucidating the penetration phenomena of the tethered chains into the mucus and its role in the polymer mucoadhesive properties.

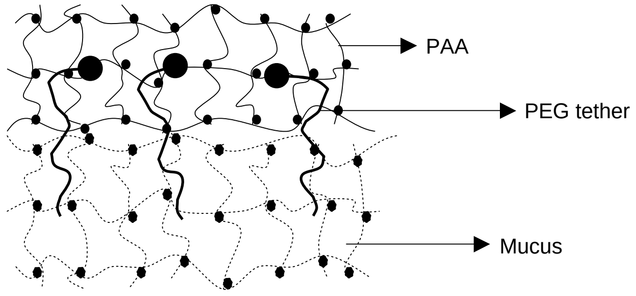

Fig. 1. Chain interpenetration of PEG tethers into the mucus.

## 2. Materials and methods

### *2.1. Synthesis of mucoadhesive hydrogels*

The monomers acrylic acid (AA) and poly(ethylene glycol) monomethylether monomethacrylate (PEGMA) with PEG molecular weight of approximately 2000, corresponding to 45 repeating units, were purchased from Aldrich Chemical Company (Milwaukee, WI). The monomer PEGMA with PEG molecular weight of approximately 1000, corresponding to 23 repeating units, was purchased from Polysciences, Inc. (Warrington, PA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Polysciences, Inc. (Warrington, PA) and used as the crosslinking agent in the amount of 0.75% mol of the total monomers. Irgacure® 184 was the photoinitiator (Ciba-Geigy Co., Hawthorne, NY). It was incorporated in 1% weight of the monomer mixture. AA was vacuum distilled at 54 °C/25 mmHg in order to remove the inhibitor, hydroquinone. Irgacure® 184, PEGMA and EGDMA were used as received. Porcine stomach type III mucin was purchased from Sigma Chemical Co. (St Louis MO).

The copolymers composed of poly(acrylic acid) (PAA) and PEG tethers were synthesized using free radical solution UV-polymerization. The monomers, AA and PEGMA of molecular weight 1000 or 2000, depending on the formulation, were dissolved in water in 1:1 weight ratio. Also, depending on each formulation the molar feed ratio between acrylic acid monomer units and ethylene glycol monomer units varied.

EGDMA was used as the crosslinking agent, and was added at 0.75% molar of the total amount of monomers. To initiate the reaction, 1-hydroxycyclohexyl phenyl ketone (Irgacure®184) was used at 1% weight of the total monomer amount. The monomer mixture was bubbled with nitrogen for 20 min, placed between two glass slides separated by Teflon spacers, and polymerized under UV light at an intensity of 12 mW/cm$^2$. The polymer films obtained were cut into discs, which were washed in deionized water for 7 days to eliminate any unreacted monomer, initiator or other impurities, and dried in vacuum oven at 27 °C for 3 days.

### *2.2. Characterization of the hydrogel films*

#### *2.2.1. Structural characteristics*

The carboxylic groups present in the acrylic acid moieties are responsible for the pH-sensitive behavior of the copolymers prepared. Since the molar ratio of acrylic acid to ethylene glycol repeating units was varied in each copolymer formulation, in order to obtain different PEG tethered designs, the percentage of moieties susceptible to ionization in the copolymer structure also varied. Consequently, the decomplexation behavior exhibited by each of the copolymer compositions was different.

Using the Henderson–Hasselbalch equation, Eq. (1), the pH transition of the different copolymers could be calculated.

$$\mathrm{pH} = \mathrm{p}K_\mathrm{A} + \log\left[\frac{\mathrm{A}^-}{\mathrm{HA}}\right] \tag{1}$$

In this equation, pH is the pH of the medium and p$K_\mathrm{A}$ is the p$K_\mathrm{A}$ value of the acid, A$^-$ is the concentration of deprotonated carboxylic groups, and HA is the concentration of protonated carboxylic groups. Knowing the p$K_\mathrm{A}$ of acrylic acid, the concentrations of A$^-$ and HA can be calculated, as they varied as a function of the pH of the environment. The percentage of ionization exhibited by a certain copolymer composition at a certain pH gives us an idea of the decomplexation and swelling characteristics of such formulation at a certain pH. This is essential information when studying the potential mucoadhesive properties of a polymeric material.

Dynamic swelling studies were performed in all formulations in buffer solutions of pH 7. Each copolymer disc was weighed in dry state and submerged in a glass container filled with 200 ml of swelling media. The experiments were carried out at 37 °C. At certain time intervals, the swelling discs were taken and weighted again to determine the water uptake as decomplexation occurred. The dynamic swelling experiments were performed at time intervals within 24 h.

Two swelling parameters were determined, the weight degree of swelling, $q$, and the degree of hydration, $H$, shown in Eqs. (2) and (3), All studies were in triplicates.

$$q = \frac{W_\mathrm{swollen}}{W_\mathrm{dry}} \tag{2}$$

$$H = \frac{W_\mathrm{swollen} - W_\mathrm{dry}}{W_\mathrm{dry}} 100 \tag{3}$$

where $W_\mathrm{swollen}$ and $W_\mathrm{dry}$ refer to the weights of the copolymer disc in the swollen and dry states, respectively.

### *2.3. Mucoadhesion experiments*

Investigation of the mucoadhesive bond strength between the surface of samples of preswollen copolymers and reconstituted mucin solutions was carried out using a tensiometric technique [27]. In a typical tensile experiment, the force, $F$, necessary for detachment of the two surfaces was recorded as a function of elongation, $l$, observed at the polymer–mucus interface [27]. The work of adhesion, $W_\mathrm{adh}$, was calculated as the area under the force-elongation curves [17,12]. The fracture energy, $\in$, is given by the reversible work of adhesion, $W_\mathrm{r}$, and the energy dissipated during the irreversible process, $W_\mathrm{i}$. Here,

$$\in = W_\mathrm{r} + W_\mathrm{i} \tag{4}$$

We may assume that due to the thermodynamic compatibility of the two surfaces in contact, polymer and mucus, the detachment process is reversible [27]. Then, $W_\mathrm{i}$ is approximately zero and we can conclude that the fracture energy, $\in$, is equal to the work of adhesion, $W_\mathrm{adh.}$

$$\in = W_\mathrm{r} = W_\mathrm{adh} \tag{5}$$

The stress, $\sigma$, is equal to the force, $F$, divided by the total initial area, $A_0$,

$$\sigma = \frac{F}{A_0} \tag{6}$$

and the strain, $\varepsilon$, is equal to

$$\varepsilon = \frac{(\delta - \delta_0)}{\delta_0} \tag{7}$$

where $\delta_0$ is the original thickness of the testing specimen and mucus, and $\delta$ is the time-dependant value of this thickness. Thus, the area under the stress–strain curve, multiplied by $A_0$ and $\delta_0$, is equal to the work of adhesion.

$$W_{\mathrm{adh}} = \sigma\varepsilon A_0\delta_0 = F(\delta - \delta_0) = Fl \tag{8}$$

In this study, the stress–strain curves were determined using a tensile apparatus (Instron series 4301). Two cylindrical metallic supports were held by each of the clamps, lower and upper, of the tensile tester. On the upper support, a polymer disc was attached, using cyanoacrylate medical adhesive. The polymer samples were previously swollen for 10 min in a PBS solution (pH 7). Reconstituted mucin solution was prepared by mixing 2% weight of porcine mucin in warm water. The solution was agitated till it had a gel-like, homogeneous appearance. The mucin solution was then placed at the lower holder of the tensile tester, and the two surfaces were put together and maintained in contact for 10 min. The upper clamp was then pulled up at a constant rate of 1.5 mm/min. Data was recorded, stress versus strain curves were plotted, and the work of adhesion was calculated, as discussed earlier.

## 3. Results and discussion

P(AA-g-EG) copolymer hydrogels with different PEG molecular weight and surface coverage were synthesized by free radical solution UV-polymerization. The kinetics of this polymerization has been studied already in the past [28–30] showing a double bond conversion approximately of 100%. The copolymer hydrogels obtained were transparent, with uniform surface, flexible and sticky. The effects of different PEG-tethered designs onto a PAA backbone were studied using a tensile technique. Also, hydrogels were characterized in order to evaluate their hydrophilic and anionic properties.

### 3.1. Copolymer structure

Two parameters contribute to the adhesion of the tethered chains to the mucosa, the surface coverage of the tethers on the polymer surface and the molecular weight or length of the tethers. Here, we synthesized mucoadhesive copolymers using AA and PEGMA. PAA exhibits very good mucoadhesive properties while PEG chains are known to be mucoadhesive promoters. The methacrylate functional groups present in the PEGMA molecules allowed the covalent attachment of the PEG chains onto the PAA backbone, and the consequent achievement of a grafted or tethered PEG structure.

In order to examine the effects of the molecular weight or length of the PEG molecule on hydrogel properties, PEG chains of 1000 and 2000 were used to decorate the PAA backbone. On the other hand, four different surface coverage designs were studied for each of the PEG type used. Different PEG surface coverages were possible by varying the molar amounts of PEG used in the polymerization; eight different tethered designs were obtained and evaluated. Fig. 2 shows a schematic representation of the PEG-tethered designs synthesized. Additionally, a polymer composed of pure PAA was synthesized in order to evaluate its mucoadhesive properties in comparison to a modified PAA. Table 1 shows the different copolymers synthesized and their compositions.

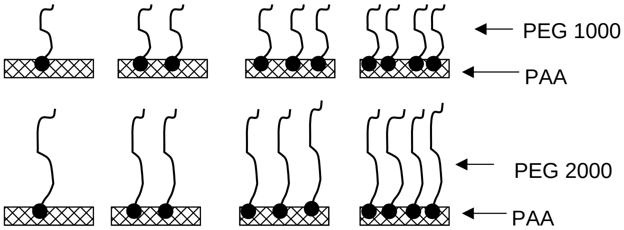

Fig. 2. Schematic representation of the PEG-tethered designs onto a PAA backbone. Four PEG surface coverages were studied for each type of PEG tether used: 1000 and 2000.

### 3.2. Characterization of the copolymer films

#### 3.2.1. Anionic properties of the copolymer films

The degree of ionization of the copolymer hydrogels was studied at different pH values, using the Henderson–Hasselbalch equation (Eq. (1)). In these analyses we considered that the copolymer was composed of a certain number of acrylic acid units, AA, and ethylene glycol units, EG, depending on the molar ratio of each formulation. Based on 100 copolymer units, involving AA and EG, we calculated how many units were ionized at a specified pH value. Note that only AA moieties in the copolymer network are able to be ionized, due to the presence of a carboxylic group in the molecule.

The results presented in Figs. 3 and 4 show the percentage of ionized units as a function of the pH, for the different copolymer designs studied. Analysis was done at pH values from 1 to 8. Since, the p$K_A$ of acrylic acid is 4.26, the ionization percentage was also analyzed at this value. In all formulations, at pH of 4.26 half of the acrylic acid groups of each copolymer were ionized.

Table 1
Chemical composition of the P(AA-g-EG) copolymers in terms of molar ratio between acrylic acid (AA) and ethylene glycol (EG)

| PEG type | AA/EG molar ratio | Surface coverage design |
|---|---|---|
| PEG 1000 | 20:80 | A |
| PEG 1000 | 40:60 | B |
| PEG 1000 | 60:40 | C |
| PEG 1000 | 80:20 | D |
| PEG 2000 | 12:88 | A |
| PEG 2000 | 25:75 | B |
| PEG 2000 | 44:56 | C |
| PEG 2000 | 67:33 | D |
| N/A | 100:0 | N/A |

Four different surface coverage designs (A, B, C, and D) were studied for each of the PEG type used. A polymer composed of pure PAA was also synthesized.

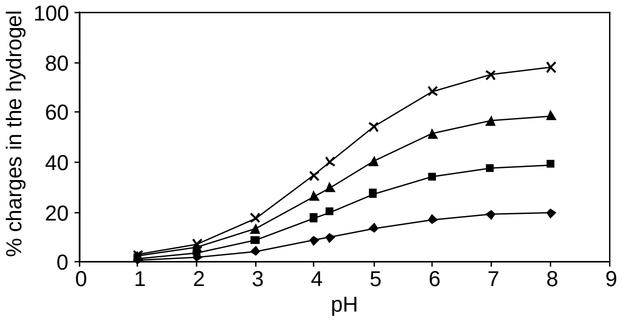

Fig. 3. Percentage of negative charges in the P(AA-g-EG) copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 1000. (20:80 AA/EG (♦) 40:60 AA/EG (■) 60:40 AA/EG (▲), 80:20 AA/EG, (⊠).

As Figs. 3 and 4 show, at pH values below 4.0 the number of negative charges was very low. The carboxylic groups of the AA moieties were hardly ionized. Then, in these cases, the PEG chains were able to interact with the non-ionized carboxylic groups, by hydrogen bonding. This phenomenon of 'Complexation' by hydrogen bonding has been discussed by us in previous work and has been well quantified. Consequently, the copolymer presented a collapsed conformation [8,7].

On the other hand, as the pH value gets closer to the p$K_A$, carboxylic acid ionization occurs leading the breakage of the hydrogen bonds, a phenomenon commonly known as 'decomplexation'. At pH values above the p$K_A$ of the acrylic acid, the number of ionized units increases dramatically. The ionization of the carboxylic groups results in a repulsive interaction between the PEG chains and the AA molecules, and is followed by copolymer decomplexation. Thus, it is at higher pH values that the PEG chains are not interacting with the poly(acrylic acid) backbone and consequently they are able to diffuse through the mucus and enhance mucoadhesion. This is the reason why in the past such systems showed higher mucoadhesive properties in basic pH values than acidic pH values [31].

### 3.2.2. Hydrophilic properties of the copolymer films

Dynamic swelling studies were performed to investigate the thermodynamic behavior of these systems in the swollen state. The dynamic water uptake in a buffer solution of pH 7 for each of the copolymer formulations studied is shown in Fig. 5–8.

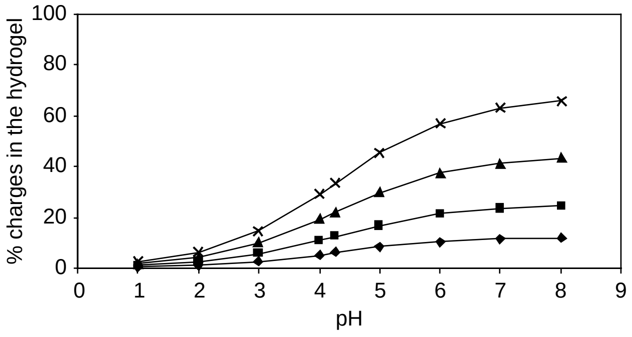

Fig. 4. Percentage of negative charges in the P(AA-g-EG) copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 2000. (12:88 AA/EG(♦), 25:75 AA/EG (■), 44:56 AA/EG(▲), 67:33 AA/EG, (⊠).

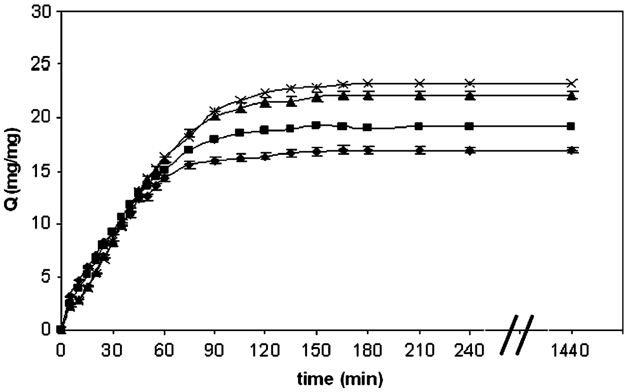

Fig. 5. Dynamic swelling behavior of P(AA-g-EG) copolymers at pH 7. Copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 1000. (20:80 AA/EG (♦) 40:60 AA/EG (■) 60:40 AA/EG (▲), 80:20 AA/EG, (⊠). $n=3$.

The different copolymer compositions resulted in different equilibrium swelling values attained after 24 h, as shown in Figs. 5 and 6. As the AA content in the copolymer composition increased, the equilibrium degree of swelling increased. This happened since higher amounts of acrylic acid in the copolymer resulted in higher amounts of negative charges present in the network at pH 7 (Figs. 3 and 4). Consequently, the degree of copolymer decomplexation by breakage of the hydrogen bonds and the associated swelling were larger in those cases due to larger ionic repulsions.

Figs. 7 and 8 show the dynamic swelling results obtained during the first 30 min. Contrary to what was observed in Figs. 5 and 6, the formulations with higher PEG content in their molecular structures exhibited larger initial water uptake. An explanation to this observation could be the highly hydrophilic properties of the PEG molecule [31]. Since, the PEG chains are very hydrophilic, formulations with higher PEG content experienced a fast initial water uptake. However, the amount

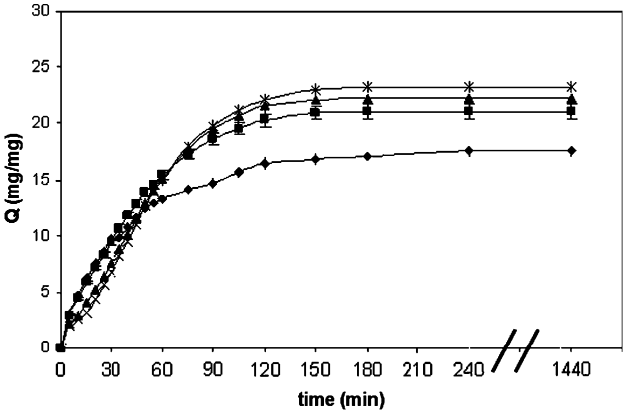

Fig. 6. Dynamic swelling behavior of P(AA-g-EG) copolymers at pH 7. Copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 2000 (12:88 AA/EG (♦), 25:75 AA/EG (■), 44:56 AA/EG (▲), 67:33 AA/EG, (⊠). $n=3$.

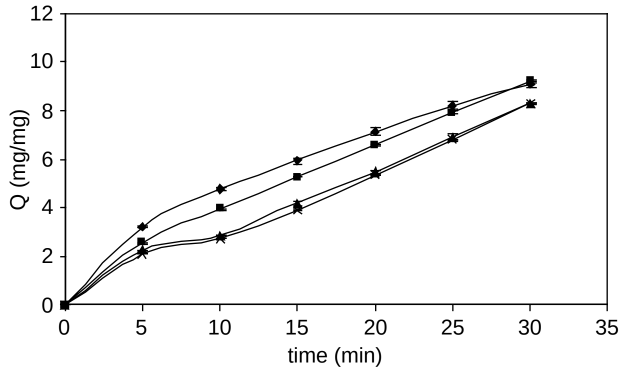

Fig. 7. Dynamic swelling behavior of P(AA-g-EG) copolymers at pH 7 during the first 30 min. Copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 1000 (20:80 AA/EG (◆) 40:60 AA/EG (■) 60:40 AA/EG (▲), 80:20 AA/EG, (⊠). $n=3$.

of water imbibed at equilibrium was determined by the final degree of hydrogel decomplexation.

During the mucoadhesive experiments, the copolymers were previously swollen in a buffer solution of pH 7 for 10 min. We found that 10 min exposure to a buffer solution is sufficient to swell the system to conditions similar to those in the upper small intestine. Also this value simulates accurately the physiological situation where the hydrogel travels through the GI tract (unswollen under the conditions described above) to the upper small intestine, target site of adhesion (where it starts swelling and decomplexing). Consequently, the amount of water imbibed by the copolymer at the time of contact with the mucus is of special interest. Fig. 9 shows the percentage of hydration of the copolymers at 10 min. Copolymers synthesized with PEG 2000 exhibited hydration of 400% for the surface coverage designs A and B, and approximately 200% hydration for the surface coverage designs C and D. Copolymers containing PEG 1000 exhibited hydration of 400%, for the surface coverage design A, and 200% for the C and D ones. However, the surface coverage design B, corresponding to the 40:60 AA/EG copolymer, exhibited a hydration of 300% at the time of contact with the mucus.

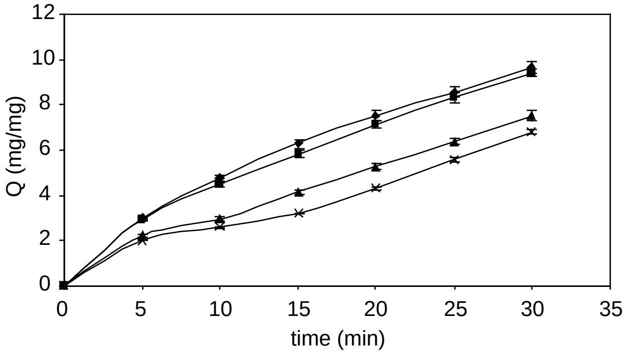

Fig. 8. Dynamic swelling behavior of P(AA-g-EG) copolymers at pH 7 during the first 30 min. Copolymers prepared with varying AA:EG molar feed ratios. Four PEG-tethered designs are shown for those networks prepared with PEG 2,000 (12:88 AA/EG (◆), 25:75 AA/EG (■), 44:56 AA/EG (▲), 67:33 AA/EG, (⊠). $n=3$.

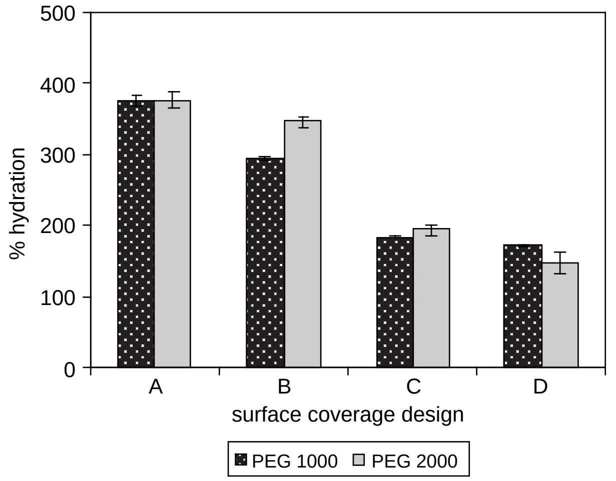

Fig. 9. Percentage of hydration of P(AA-g-EG) copolymers at pH 7 during the first 10 min of swelling. Copolymers prepared with varying AA:EG molar feed ratios. Four different surface coverage designs (A, B, C, and D) were studied. $n=3$.

### 3.3. Mucoadhesion experiments

During the mucoadhesion experiments, different PEG-tethered designs were studied. Our objective was to improve the mucoadhesive performance of a crosslinked poly(acrylic acid), a well-known mucoadhesive polymer, by decorating it with PEG tethers. For the mucoadhesion experiments only the results concerning copolymers with PEG 1000 tethers will be shown here. PEG 2000 formulations need to be further studied regarding their mucoadhesive characteristics. As shown in Fig. 10 and Table 2, formulations containing higher amounts of PEG 1000 tethers in their structures, 20:80 and 40:60 AA/EG, showed larger values for the work of adhesion, 40:60 AA/EG being the largest. In order to discuss these results, it is important to mention some of the earlier work done in our laboratories. We found that when a polymer system comes into contact with the mucus, the concentration gradient across the interface causes the PEG chains to diffuse out of the network and penetrate into the mucus layer. This interpenetration phenomenon is believed to increase the adhesion of the polymer to the mucus [27,20,22]. However, as observed in Fig. 10 and Table 2, different PEG tethered structures onto the PAA backbone affected differently the mucoadhesive performance of the copolymer. Then, it is not only the presence of tethered chains necessary to promote mucoadhesion but also their disposition onto the backbone has a great influence on this phenomenon. The application of the single-chain mean filed (SCMF) theory [25,24] can help us understand the results obtained. According to this mathematical theory, it is important to consider the repulsion forces between the tethered chains among themselves and the PAA backbone. At low surface coverage, i.e. at low amounts of PEG tethers covalently attached to the PAA backbone, the grafted chains are situated outside of the hydrogel. However, as we increase the surface coverage, the repulsion between the tethers among themselves also increases, thus provoking the displacement of some of the tethers inside the hydrogel. As a consequence, the tethers

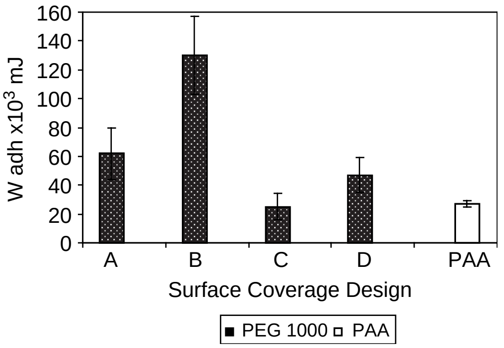

Fig. 10. Work of adhesion values obtained in the tensile experiments for the P(AA-g-EG) copolymers containing PEG 1000. Copolymers were prepared with varying AA:EG molar feed ratios. Four different surface coverage designs (A, B, C, and D) were studied. A polymer composed of pure PAA was also studied. $n=4$.

available for the interaction with the mucus are decreased, resulting in a lower polymer-mucus interaction.

In our case, the copolymer formulations 20:80 and 40:60 AA/EG contain a dense PEG 1000 tethered design. Then, the interpenetration of the PEG tethers promotes and reinforces the adhesion of the copolymer to the mucus. However, the formulation 20:80 AA/EG showed a lower value of work of adhesion than the 40:60 AA/EG formulation. An explanation to this observation is that the formulation 20:80 AA/EG probably has too dense of a PEG tethered structure onto the PAA backbone, resulting in a repulsive interaction between PEG chains among themselves. As a consequence of this phenomenon, some of the tethers are displaced inside the backbone of the polymer and thus fewer PEG tethers are actually available at the interface for interpenetration into the mucous layer.

The copolymers 60:40 and 80:20 AA/EG have lower amounts of PEG 1000 tethers in their molecular structure. The PEG-tethered coverage designs in these formulations are sparser. Although their tethers are unlikely to experience a repulsion interaction between themselves, the number of chains available to promote the mucoadhesion phenomenon is less than the more densely PEG tethered designs. Then the mucoadhesive capabilities shown are reduced. However, it is very important to understand that in all cases, there exists a synergistic effect of both polymers constituting the hydrogel: the PAA backbone, which provides hydrogen bonding between the hydrogel and the mucous layer, and the PEG tethers, which contribute to the mucoadhesion phenomenon by reinforcing chain interpenetration. Both phenomena will contribute to the performance of the copolymer and to their mucoadhesive properties.

Table 2
Work of adhesion values obtained in the tensile experiments for the P(AA-g-EG) copolymers

| MW PEG | AA/EG | Design | $W_{\mathrm{adh}}\times10^3$ mJ |
|---|---|---|---|
| 1000 | 20/80 | A | 62±18 |
| 1000 | 40/60 | B | 130±27 |
| 1000 | 60/40 | C | 25±9 |
| 1000 | 80/20 | D | 47±12 |
| N/A | 100/ 0 | N/A | 27±2 |

Copolymers were prepared with varying AA:EG molar feed ratios. Four different surface coverage designs (A, B, C, and D) were studied. A polymer composed of pure PAA was also studied. $n=4$.

In conclusion, our results show that the addition of covalently attached PEG 1000 tethers onto the PAA improved in certain cases the mucoadhesive performance of a not molecularly modified PAA polymer when the suitable tethered structure was attained. It was found that at high PEG surface coverage the mucoadhesive capabilities of the hydrogel were considerably increased, although it was observed that too dense PEG-tethered designs were not desirable. Consequently, the formulation 40:60 AA/EG appeared to be the best mucoadhesive copolymer of the different molecularly designed networks studied. This formulation showed a 480% increase on the work of adhesion value in comparison to a polymer composed of pure PAA (Table 2).

On the other hand, as mentioned before, the first step necessary for the formation of a mucoadhesive bond between a polymer and the mucus is the wetting and swelling of the polymer. This step is crucial since the amount of water imbibed by the polymer at the time of adhesion will affect the interaction of the system with the mucous at the interface. The different chemical composition of each copolymer studied resulted in different polar and hydrophilic properties (Fig. 5–9). Depending on the AA:EG molar ratio, a copolymer was more or less likely to imbibe an aqueous phase in the network. During the mucoadhesion experiments, the copolymer samples were previously swollen for 10 min in a buffer solution, pH 7, in order to simulate physiological conditions, as discussed above. Depending on the hydration attained after this period, the thermodynamics involved in the mucoadhesion process was different. Thus, we believe that the degree of swelling could influence the performance of the copolymers. Fig. 9 shows hydration of the different copolymers attained at 10 min. The copolymer that had shown the highest value for the work of adhesion, 40:60 AA/EG, presented a hydration of 300% at the time of contact with the mucus. The 20:80 AA/EG formulation showed a 400% hydration at 10 min and formulations 60:40 and 80:20 AA/EG presented values of hydration approximately of 200%. According to these results, an intermediate degree of hydration appeared to be more desirable to assure a proper mucus–hydrogel interaction.

## 4. Conclusions

In this research, different PEG-tethered structures on acrylic-based polymers were designed and studied in order to improve the mucoadhesive properties of a pure crosslinked poly(acrylic acid). A maximum mucoadhesion could be achieved under some optimal tethered structures and copolymer compositions. Current acrylic-based mucoadhesive materials were improved by grafting appropriate amounts of PEG 1000 tethers. As previous theoretical calculations previewed, certain tethered structures optimized mucoadhesion, thus an improved drug delivery carrier was obtained.

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