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Permeability studies in chitosan membranes. Effects of crosslinking and poly(ethylene oxide) addition

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Abstract—Pure chitosan, glutaraldehyde crosslinked chitosan, and a blend of chitosan with poly(ethylene oxide) (PEO) membranes were prepared. The three membranes were characterized in terms of their swelling capacities as well as their permeabilities to a drug model (sulfamerazine sodium salt). For the permeation experiments, the variables analyzed were the type of membrane and the initial drug concentration in the liquid phase (from 0.1% to 1.5%). Permeability coefficients were calculated using UV spectroscopy. The results showed that for the three analyzed membranes, the permeability did not change with time (over the studied time interval). An increase in the permeability for CHI/PEO membranes compared to those made of pure chitosan was also observed, possibly due to microporous region formation and/or crystallinity reduction. For the crosslinked membrane, an even higher increase in the permeability coefficient was observed. In this case, the increase was attributed to free volume variation.

Keywords: Chitosan; Glutaraldehyde; Permeability; Sulfamerazine sodium salt

1. Introduction

The history of chitosan began in 1859 when Rouget discovered the deacetylated form of chitin.[1] Over the last 20 years, a huge amount of work has been published about this polymer and its potential applications. Chitosan has low toxicity, is biodegradable, and biocompatible.[2–5] The presence of amino groups makes it soluble in dilute acidic solutions. Moreover, because of the D-glucosamine groups, the pK_a is about 6.5 and thus the polymer is positively charged in acidic solution.[6] Being a polycation, chitosan can form electrostatic complexes with negatively charged species like proteins, anionic polyelectrolytes, drugs, and low molecular weight anions.[7,8]

Due to its properties such as mucoadhesivity and biocompatibility, among others, there has been a growing interest in pharmaceutical industry in the use of chitosan as a drug carrier in different forms including membranes, micro- and nanoparticles, gels, tablets, and capsules.[2,7,9,10] For example, some transdermic systems based on this polymer were obtained for controlled release of niphedipine and propanolol.[11–13] It was also found that chitosan membranes can be used as a skin-imitating barrier in the evaluation of transdermal drug delivery systems and in routine quality control tests of these drug delivery systems.[14]

1.1. Chitosan/poly(ethylene oxide) (PEO) blends

Modification of existing polymers has developed greatly over the past decade because the time and costs involved are smaller than those necessary to obtain new polymers. A blend can be defined as a mixture prepared from at least two structurally different polymers that interact without covalent bond formation.[15] One of the main advantages of this approach is that materials with different properties from the original polymers are obtained. The blend can be formed by different methods: physical mixture of two polymers under fusion conditions,

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dissolution in a common solvent with subsequent evaporation, or in situ polymerization where monomer polymerization occurs in the presence of a polymer.

In the present paper, the poly(ethylene oxide) (PEO) choice was based on characteristics such as its low toxicity and water solubility. Besides, due to the existence of C–O bonds in its structure, this polymer can complex with others including chitosan. PEO can thus act as a proton acceptor while chitosan can act as a proton donor, giving rise to homogeneous polymeric blends. Moreover, PEO has been shown to effectively increase the blood compatibility of different polymeric materials, including chitosan.[8,16–18] To use membranes in several controlled release applications, it is necessary to have an understanding of the swelling characteristics and permeability to model drugs. Therefore, in this work, we investigate the relationship between the membrane PEO content and properties like swelling capacity and permeability to the sodium salt of sulfamerazine. The major advantages of this approach are its simplicity, low cost, and improved biocompatibility due to the presence of the PEO.[16,19]

1.2. Crosslinked chitosan

Due to its excellent biocompatibility, chitosan has been widely used in biomedical applications. However, chitosan has some drawbacks: it is soluble in acidic media (pH <4.5) and its mechanical properties are not good enough for some applications. Therefore, the crosslinking reaction was used in an attempt to not only reduce membrane solubility but also change its permeability in relation to a model drug (sodium sulfamerazine).

Crosslinking is a process where polymeric chains are linked either by covalent bonds, via a chemical reaction, or by strong physical interactions (e.g., ionic interactions) between the polymer and the crosslinking agent or between two different polymers. A crosslinking reaction aims to modify the polymer molecular properties, for example, to increase chemical stability and stiffness, to alter permeability or color, or to change chelation efficiency, protein absorption capacity, and thermal stability, among others. The most common chitosan crosslinking agents are the dialdehydes like glyoxal and glutaraldehyde. Glutaraldehyde is a very reactive species, being able to undergo polymerization in aqueous media. The glutaraldehyde reaction with compounds that have primary amino groups is well known and has been extensively used, either to identify the presence of free primary amino groups or to obtain more complex molecules.[20–22] The precise crosslinking reaction mechanism is not well defined yet. However, reports[12,23,24] indicate that variables like chitosan and glutaraldehyde concentration, degree of deacetylation, solvent, pH, and reaction temperature must be carefully considered, because they can change the physical and chemical properties of the final product.

2. Materials and methods

Chitosan was supplied by Polymar (Brazil) with a degree of acetylation of 15% according to the supplier. Polyethylene oxide (PEO) was purchased from Sigma (USA) with a MW = 1,000,000 g/mol. All other chemicals were analytical grade reagents and were used as received.

2.1. Membrane preparation

2.1.1. Pure chitosan. Chitosan was dissolved by stirring in aqueous acetic acid solution (2.0 wt %) for 24 h. The resultant solution was filtered using a Millipore Millex filter, casted on a glass plate and dried in oven at 50 °C for 12 h. The membrane was neutralized by immersion in a 1.25 M sodium hydroxide solution for 2 h and then repeatedly washed with distilled water and allowed to dry at room temperature.

2.1.2. Crosslinked chitosan. Water-conditioned chitosan membranes were treated with 0.01% (w/v) glutaraldehyde (Vetec-Brazil) solution for 48 h at room temperature. The solution pH was 9.5–10 and the membranes then were rinsed with distilled water and allowed to dry at room temperature.

2.1.3. Chitosan/PEO blend. PEO was dissolved by stirring in an acetic acid aqueous solution (2.0% v/v) for 24 h, providing a PEO solution (1.0% w/v). An appropriate amount of chitosan solution, prepared as previously described, was added to the PEO solution at a ratio of 95:5, and the mixture was stirred for 1 h. The solution was then casted on a glass plate, dried in an oven, rinsed, and allowed to dry in exactly the same way as for pure chitosan.

All dry membrane thicknesses were measured with a digital micrometer (Check Line DCF 900 USA). Each membrane was measured at 10 different points (5 points at each side) and only those with a relative standard deviation smaller than 10% were used. The membrane thicknesses were in the range of 30–40 μm. Pure chitosan membranes, the crosslinked membranes, and those blended with PEO are named CHI, CHI/CG, and CHI/PEO, respectively.

2.2. Swelling experiments

Membranes were immersed in distilled water at room temperature. At regular time intervals, they were removed from the water, dried with a toilet tissue, and weighed on an analytical balance B-TEC-U210A

Table 1. Calculated water sorption percentage

	Water sorption (%)	
	$t = 12$ h	$t = 48$ h
CHI	100.2 ± 1.6	94.2 ± 1.4
CHI/PEO	102.1 ± 0.7	102.1 ± 0.8
CHI/CG	99.0 ± 1.4	100.1 ± 0.7

(TECNAL-Brazil). The water uptake was calculated according to Eq. 1:

$$\% = \frac{m_w - m_d}{m_d} \times 100 \quad (1)$$

where m_w and m_d are the membrane wet and dry weight, respectively. Five samples were used for each type of membrane. Table 1 presents the calculated average values and their standard deviation for an immersion period of 12 and 48 h.

2.3. Permeation experiments

Permeation experiments were carried out in a thermostatic bath at a temperature of 30.0 ± 0.1 °C, using a system described elsewhere,[25] consisting of two cylindrical half cells 230 mL in volume, submitted to constant stirring and separated from each other by the chitosan membrane. The 8.55 cm^2 membrane area was not supported. All membranes were immersed in distilled water for 12 h before the experiment. All experiments were done in duplicate.

The feed solution was prepared by dissolution of sodium sulfamerazine salt (solubility = 1 g/36 mL of water at 25 °C, MW = 286.3, pH = 7.0)[26] in distilled water. The choice of the drug was done taking into account its water solubility, pH, and suitability for ultraviolet (UV) absorption. The drug concentrations used were 0.1%, 0.5%, 1.0%, and 1.5%. The receiving solution was distilled water and the amount of solute that permeated through the membrane was determined using a Varian UV spectrometer ($\lambda = 260$ nm). Every 10 min, a 2.5 mL sample was taken from the receiving solution, analyzed, and then returned to its compartment. All experiments were done in duplicate.

Permeability coefficients (P) were calculated as described by Crank[27] for diffusion through a membrane. The method has already been fully described in the literature when used to calculate the permeability of amitriptiline and sodium isoniazide salts through chitosan membranes.[28] Briefly, one can assume that the membranes initially had a null drug concentration ($c_0 = 0$) and that the drug concentration on one face was much higher than the one at the emerging face (i.e., $c_1 \gg c_2$) and, therefore, $c_1 - c_2 \approx c_1$. In this case, the total amount of diffusing substance (Q_t), passing through the membrane over time t, per unit of area, is given by Eq. 2 as follows:

$$Q_t = \frac{Dc_1}{L}\left(t - \frac{L^2}{6D}\right) \quad (2)$$

where D is the diffusion coefficient and L is the hydrated membrane thickness.

Because the amount of drug that passed through the membrane was measured spectroscopically, Q_t can also be given by

$$Q_t = \frac{VA}{\varepsilon bS} \quad (3)$$

where V is the half cell volume, A is the absorbance, ε is the drug extinction coefficient, b the UV cell pathlength, and S the membrane area. Substituting Eq. 3 into Eq. 2 and substituting D by P/K, where K is the partition coefficient, it follows that

$$A(t) = \frac{PC_1\varepsilon bS}{VL}t - \frac{c_1 L\varepsilon bS}{6V} \quad (4)$$

where C_1 is the initial solution concentration. Eq. 4 shows that the angular coefficient of the curve generated by the plot of absorbance versus time can be used to calculate the membrane permeability coefficient.

3. Results and discussion

3.1. Swelling experiments

The weight gain resulting from membrane water sorption was measured by immersion in distilled water for different time intervals. Table 1 shows water uptake percentage, at 12 and 48 h, for the three analyzed membranes. As shown in the table, the water sorption capacity after 12 h is very similar for CHI and CHI/CG and shows a slight increase for CHI/PEO sample. Such increase in hydrophilicity could be caused by the OH hydrophilic groups present in PEO. These results are in good agreement with previous ones obtained by thermal analysis.[29] The 48 h experiment was carried out to check if sorption equilibrium was already reached after 12 h (immersion time used before the permeability experiments). The results show that for CHI/CG and CHI/PEO membranes, the values are nearly constant, indicating that equilibrium values were established. However, for CHI membranes the water sorption tended to decrease. This weight loss could be associated with a process known in the literature as erosion,[18] which means that, although chitosan is not water soluble, at longer times a small amount of this material could be solubilized, causing the observed weight loss. For the CHI/PEO and CHI/CG samples, this process is suppressed either by physical interactions in the case of CHI/PEO or by additional covalent bonds in the case of CHI/CG.

3.2. Permeation experiments

The results from the permeation experiments with sodium sulfamerazine solution, at different concentrations, using pure chitosan membranes are plotted in Figure 1. Similar results for CHI/PEO and CHI/CG membranes are shown in Figures 2 and 3, respectively. The experiment time was limited by the absorbance values, that is, the experiment was interrupted as soon as the sample collected from the receiving solution reached an absorbance value next to 2.0.

As can be seen from Figure 1, one can observe the absence of time lag that, according to the literature[27,30] can be defined as the time necessary for the system to reach its steady state. This fast steady state establishment can be attributed to two factors: the small membrane thickness and the fact that the membrane was previously hydrated before the experiment, which eliminates the swelling step during the permeation experiment. The linear behavior over the concentration range analyzed is notable and allows us to calculate the permeability coefficient from the curve angular coefficient.

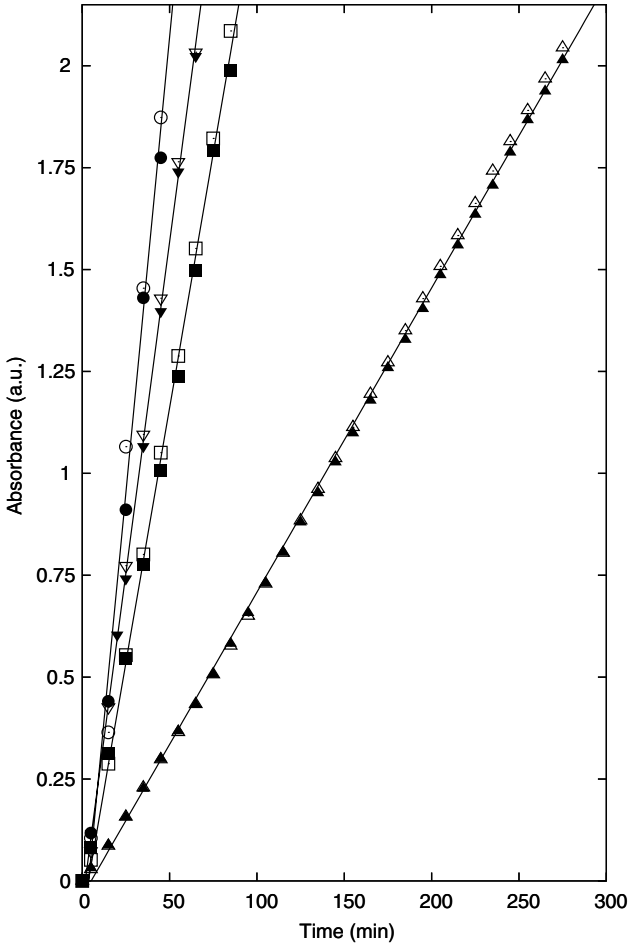

Figure 1. Plot of absorbance as a function of time for the permeation of sulfamerazine sodium salt solution through CHI membranes. Solution concentration: 0.1% (open and closed up triangles), 0.5% (open and closed squares), 1.0% (open and closed down triangles), and 1.5% (open and closed circles).

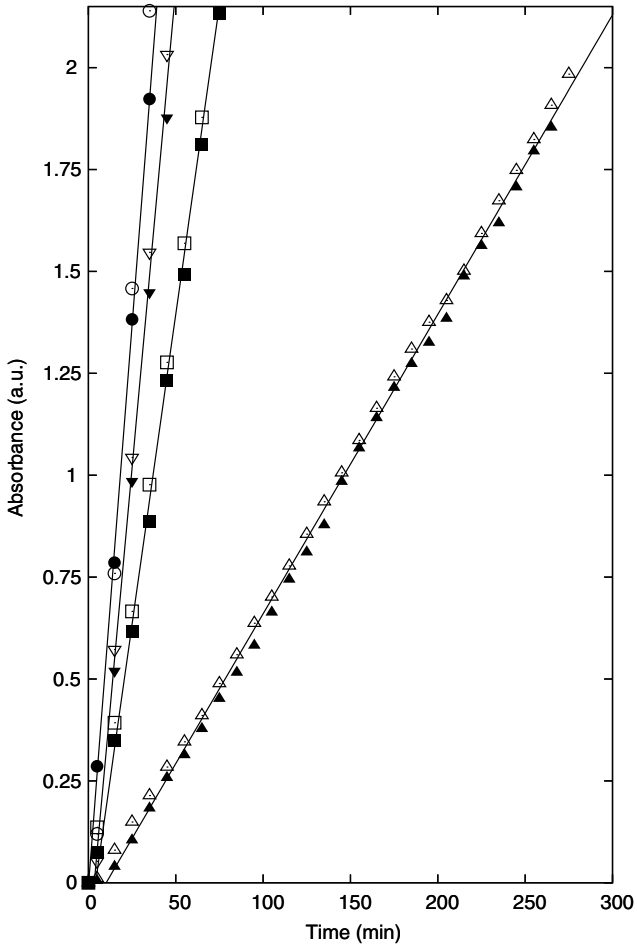

Figure 2. Plot of absorbance as a function of time for permeation of sulfamerazine sodium salt solution through CHI/PEO membranes. Solution concentration: 0.1% (open and closed up triangles), 0.5% (open and closed squares), 1.0% (open and closed down triangles), and 1.5% (open and closed circles).

When the permeability coefficient does not change with time (for the time range studied), one can say that there are no strong membrane–drug interactions. According to the literature, any interaction between the solute and the membrane will produce deviations from the linear behavior.[31] Another important observation is the increase in drug flux with increasing drug concentration, which is indicated by an increase in the angular coefficient of the resulting curves. As detailed in Figure 1, such variations of angular coefficients are bigger at lower concentrations and, as the drug concentration increases, the variations become smaller and probably tend to an equilibrium value. One should point out that such a flux increase does not necessarily indicate an increase in permeability.

Table 2 shows the calculated permeability coefficients for all analyzed membranes. One can observe a decrease

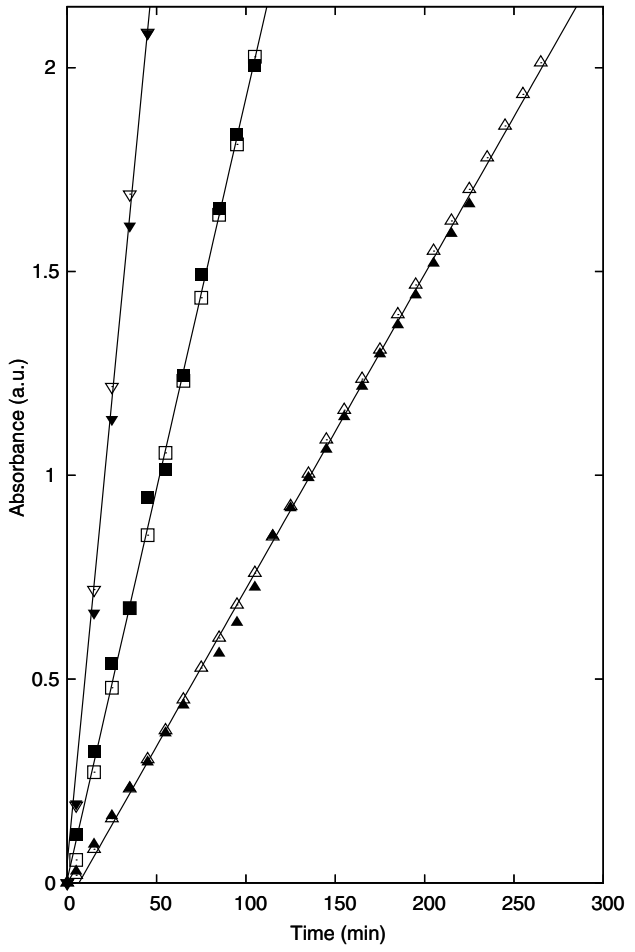

Figure 3. Plot of absorbance as a function of time for permeation of sulfamerazine sodium salt solution through CHI/CG membranes. Solution concentrations: 0.1% (open and closed up triangles), 0.5% (open and closed squares), and 1.0% (open and closed down triangles).

Table 2. Calculated permeability coefficients for CHI, CHI/PEO, and CHI/CG membranes to sulfamerazine sodium salt

Drug concentration (g/mL)	$P \times 10^5$ cm^2 min^{-1}		
	CHI	CHI/PEO	CHI/CG
0.001	1.86 ± 0.04	1.87 ± 0.10	2.72 ± 0.06
0.005	1.32 ± 0.05	1.71 ± 0.12	1.53 ± 0.23
0.010	1.00 ± 0.05	1.42 ± 0.08	1.60 ± 0.11
0.015	0.96 ± 0.02	1.30 ± 0.08	—

in permeability with increasing concentration as well as a tendency to an equilibrium value where an increase in drug concentration will no longer influence membrane permeability. This is strong evidence that the diffusion mechanism is not Fickian for the concentration range analyzed. To have a better understanding of these results, it should be remembered that Eq. 4 is used in the permeability calculation. According to this equation, the permeability value is proportional to the angular coefficient but inversely proportional to drug concentration. In this work, the drug concentration used ranged from 0.001 to 0.015 g/L so that concentration variations were higher than the angular coefficient variations.

According to the literature, the desired characteristics of a drug delivery system (DDS) are strongly dependent on four factors (the 4D's approach):[32] drug (MW, dose, polarity, stability, etc.), disease (acute, chronic, severity, etc.), destination (systemic, local, oral, etc.) and delivery (onset, duration, continuous, pulsed, etc.) So according to the 4D's, one can have cases where the zero release approach (a constant release of drug independent of concentration and drug loading in the DDS) has proved effective, and others, where, due to varying absorption rate and drug stability in different regions of the gastrointestinal tract (GIT), the drug may not achieve constant plasma levels and, in this case, the DDS must be based on a first order drug-release kinetics.[33] The results with these chitosan membranes showed that this material can present a flux that does or does not change with the concentration depending on the drug concentration used, which makes it a very versatile material for DDS.

Figure 2 shows permeation results for CHI/PEO membranes. In this case, one also observes a linear behavior for all analyzed concentrations. Likewise, CHI/PEO membranes also initially present a drug flux increase with increasing drug concentration and then tend to an equilibrium value. Figure 2 and Table 2 show that the CHI/PEO membrane permeability coefficient decreases with increasing drug concentration, as already observed for CHI membrane.

Comparing the permeability coefficients for CHI and CHI/PEO membranes, one can see from Table 2 that PEO addition tends to increase membrane permeability. To understand this increase in permeability, we can consider three possibilities: (a) the PEO addition, in solution, inhibits the chitosan re-crystallization process and, as a consequence, the blended material is less crystalline than pure chitosan; (b) because PEO is a water-soluble polymer, it could be partially removed from the blend during the washing process. As a consequence, the membrane free volume would increase or even some microporous regions could be formed[16] in contrast with the dense pure chitosan membrane, and (c) the PEO addition increases the membrane hydrophilicity. Although the swelling experiment is probably not sensitive enough to measure this possibility, previous thermogravimetric analysis (TG, DTG, and DSC)[29] has shown such an increase in hydrophilicity. In any case, the main consequence of the above mentioned possibilities would be an increase in membrane permeability.

Like CHI and CHI/PEO, the drug permeation through CHI/CG membrane also presented a linear behavior. Comparing the permeability coefficients shown in Table 2 one can see that CHI/CG membrane is more permeable that CHI and CHI/PEO membranes.

In this case, however, as shown on swelling experiments, the hydrophilicity variation is not significant and, therefore, should not be responsible for the permeability increase, as in the CHI/PEO case. One important experimental observation that can help to elucidate this increase in permeability is that the CHI/CG membrane, in contrast to the CHI and CHI/PEO membranes, presented a very significant increase in diameter when hydrated while CHI and CHI/PEO only presented thickness variations. Considering that the same volume of solution was used to produce all membranes (CHI, CHI/PEO, and CHI/CG) and that the area inside the permeability cell is the same, one can assume that in the CHI/CG membrane there will be a small number of molecules occupying the same cell area and therefore one can conclude that such membrane has a 'looser' or more opened structure. This increase in free volume could be responsible for the increase in permeability. Another observation is that the permeability coefficient for the higher drug concentration experiment (0.015 g/mL) was not measured because usually after some minutes, the membrane lost its integrity and therefore the experiment was interrupted. This is another indication that the crosslinking process has changed membrane structure resulting in a material with lower mechanical resistance.

This is a very surprising result for a crosslinked material. However, thermal analysis for this same membrane, like decrease in thermal stability[29] and other properties[12,21–24] (e.g., mechanical deterioration) confirm that when the degree of crosslinking is quite low, such as the one used in this work, the results can be very different from those expected for a crosslinked material. The effect of low crosslinking degree was already extensively discussed elsewhere.[29] In brief, it is possible that the addition of a small amount of glutaraldehyde could promote chemical modifications in the polymer chains that reduce the existing attractive interactions between chitosan molecules in the neighborhood of crosslinking points. Another possibility is the intramolecular reaction of glutaraldehyde with chitosan which could interfere on previously existing hydrogen bonds, reducing them and, consequently, increasing membrane's permeability.

4. Conclusions

The CHI/CG membrane did not present significant changes in weight gain when compared with CHI membrane. At longer times, a small amount of chitosan solubilization may occur, giving rise to an erosion process that is responsible for the weight loss observed in CHI membrane. Over the same period of time, CHI/PEO and CHI/CG tend to reach an equilibrium state with no weight variations. The smaller solubility of CHI/PEO and CHI/CG was attributed to physical interactions (CHI/PEO) as much as new covalent bonds formed (CHI/CG).

Permeability experiments showed that a steady state was reached very quickly and that the permeability coefficient does not change as a function of time (for the time interval analyzed). A decrease in permeability coefficient values as a function of drug concentration was initially observed and then, at higher concentrations, a tendency to an equilibrium value. The time independent drug permeability, which is connected with the asymptotic permeability tendency at higher concentrations, is an ideal characteristic for using such membranes in drug delivery systems. PEO addition tends to increase membrane permeability. The low crosslinking degree used in this work was responsible for a permeability increase. However, here this behavior cannot be related to an increase in hydrophilicity as shown by swelling experiments. So, in this case, structural modifications resulting from the crosslinking reaction are responsible for the permeability increase.

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