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Swelling behavior of chitosan/pectin polyelectrolyte complex membranes. Effect of thermal cross-linking

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Summary

Membranes of the polyelectrolyte complex between chitosan and pectin were prepared by precipitating the complex from a mixture of both polysaccharides. It was shown that the swelling kinetics of these membranes follows a Fickean behavior. The membranes were heated at 120ºC in order to convert the $–NH_3^+ \, ^-OOC$- salt bonds into amide bonds. The thermally treated membranes were stable in strongly acid and basic media. The extent of amide bond formation was followed by FTIR spectroscopy. It was found that as the reaction time increased, both the absorbance ratio A_{1744}/A_{1082} and the maximum swelling of the membrane decreased. The surface morphology of the membranes did not vary appreciably with the thermal treatment.

Introduction

Polyelectrolyte complexes (PEC) result from the interaction between macromolecules carrying opposite charges [1]. They present a wide range of applications, such as encapsulation of substances, drug delivery systems, and waste-water treatment, among others. The PECs made from natural ionic polysaccharides are generally non-toxic and biocompatible, properties that are very valuable for their use in medicine and pharmacy. In dependence of their final use, PECs can be prepared as powders, membranes, sponges, fibers, gels, spheres or in solutions.

Chitosan is a biopolymer composed by units of β(1-4) D-glucosamine and β(1-4) N-acetyl-D-glucosamine. Due to its unique cationic character chitosan interacts with polyanions giving rise to PECs showing specific properties. Chitosan has been used for the preparation of various polyelectrolyte-complex products with natural polyanions as carboxymethylcellulose, alginic acid, dextran sulfate, carboxymethyl-dextran, heparin, carrageenan, pectin and xanthan [2, 3].

Peptic substances are a group of polysaccharides that can be found in the cellular walls and intercellular regions of the superior plants [4]. They are composed of linear chains of α(1-4) linked polygalacturonic acid units which can be esterified with methanol. These polysaccharides are used in drugs production, such as anti-diarrheics, detoxicants and as protectors of the gastrointestinal tract [4]. In the recent years they have been studied in systems for the sustained release of substances [5, 6].

Membranes from polyelectrolyte complexes could be readily prepared from insoluble stoichiometric PECs. These membranes swell considerably in water and their diffusion properties are strongly dependent on pH, temperature, ionic strength, molar ratio of reacting polyelectrolytes among others [7-10].

Chitosan/pectin PEC membranes exhibit pH dependent swelling response [11] which is an attractive property for drug delivery systems. Chitosan/pectin/HPMC mixtures have been proposed as a film coating system for tablet cores, capable of achieving bimodal drug delivery [12] and composite matrices comprising linseed Na-pectate, alginate and chitosan have been successfully used for encapsulating shark liver oil [13].

The aim of the present work was to study the swelling behavior of membranes of the chitosan/pectin PEC in order to investigate the effect of thermal cross-linking on modulating their swelling capacity and increasing their stability at low and high pH.

Experimental Part

Materials

Chitosan from lobster cephalothorax was prepared in our Laboratory. Its viscosity average molecular weight was 1.27×10^5 estimated at 25°C in 0.3 M acetic acid/0.2 M sodium acetate [14] and the degree of *N*-acetylation (DA = 0.18) was determined potentiometrically.

Pectin from fruits was purchased from Sigma Chemical Co. It had the following composition: degree of esterification of galacturonic units (DE = 59.2%) and galacturonic acid content (GA = 40.7%) both determined by conductimetric titrations [15]. Its average molecular weight was 3.74×10^4, as estimated by viscometry at 25°C in 0.1 M NaCl [16].

Prior to use, both polymers were purified as described below. Experiments were carried out with twice distilled water. The rest of the reagents were analytical grade.

Methods

Purification of chitosan

Chitosan (5 g L^{-1}) was dissolved in 1% (v/v) acetic acid and successively filtered through glass wool, sintered glass filters (pore diameters: 45 and 5 μm) and membranes (0.8 and 0.45 μm). Then it was precipitated by drop-wise addition of 1 M NaOH until pH ≈ 9, carefully washed with water until no change in conductivity was detected and further washed with ethanol at concentrations 70, 80, 90 and 100% (v/v). The purified polymer was finally dried in vacuum at room temperature.

Purification of pectin

An aqueous 2.5 g L^{-1} solution of pectin was successively filtered through glass wool, sintered glass filters (pore diameters: 45 and 5 μm) and membranes (0.8, 0.45 and 0.2 μm). The filtered solution was passed throughout an ionic exchange resin Amberlite IR120 (H^+ form), after which the solution was neutralized up to a pH near 8 with 1 M NaOH in order to convert the polyacid to its salt form. NaCl was added to reach a final 0.1 M concentration. Then, the polysaccharide was precipitated by gradual addition of ethanol until a final 70% (v/v) alcohol concentration was attained and washed several times with the same water/ethanol mixture until no change in conductivity was detected. The solid was further washed with ethanol/water mixtures of increasing concentration: 80, 90 and 100% (v/v). The purified polymer was vacuum dried at room temperature.

Preparation of pectin/chitosan polyelectrolyte complex membranes
Chitosan was dissolved in 3 M acetic acid at 1% (w/v) concentration. At the same time, pectin was dissolved in water at 2% (w/v) and an excess of concentrated hydrochloric acid was added. Both solutions were left to stand overnight. Then, both solutions were mixed with stirring. The mixture was neutralized with 10% (w/v) NaOH until pH 5.5 giving rise to a suspension of PEC particles. The mixture was poured on a sintered glass filter. The PEC particles trapped in the glass filter were left to decant and dry for a few days. As a result of this the PEC membrane was generated.

Swelling measurements
The dry membranes were weighed periodically after carefully wiping their surfaces with a filter paper. Measurements were carried out in different media at 25°C. The degree of swelling was calculated using equation (1).

$$M_t = \frac{W_t - W_0}{W_0} \tag{1}$$

Here W_0 is the weight of the dry membrane ($t = 0$) and W_t is the weight of the swollen membrane at time t. Experiments were run in triplicate; the reported value is the average M.

Cross-linking of the PEC membranes
The membranes were placed inside capped essay tubes under nitrogen atmosphere and then heated at 120°C for different times: 30, 60, 120 and 180 minutes.

Fourier Transform Infrared Spectroscopy
Infrared spectra were registered in an FTIR Nicolet spectrometer model Protégé 460 E.S.P., Nicolet Instrument Corp., Madison, WI. The transmission spectra were collected at 2 cm^{-1} resolution and 64 scans. The reference background was air.

Scanning Electron Microscopy
The surface morphology of the membranes was observed using an environmental scanning electronic microscope (ESEM Phillips XL30).

Results and Discussion

By mixing both the chitosan and pectin solutions under strongly acid conditions it is possible to obtain a homogeneous solution without ionic cross-linking between both polyelectrolytes. The PEC is then obtained by adjusting the pH to 5.5 by addition of 10 wt-% NaOH solution. PEC formation can be schematically represented as follows:

$$\sim\!\sim COOH + {}^{+}NH_3\!\sim\!\sim \rightleftharpoons \sim\!\sim COO^{-}\,{}^{+}NH_3\!\sim\!\sim + H^{+} \tag{2}$$

The extent of this reaction is dependent on the pH of the medium.

Swelling behavior of the PEC membranes in water

PEC membranes are hydrophilic and they swell considerably in water, reaching a maximum swelling degree after a relatively short time. The process is diffusion controlled exhibiting a Fickean behavior.

In order to fit the experimental data of swelling kinetics the mathematical solution of the Fick's differential equation proposed by Crank (equation (3)) was used. This expression is referred to a flat film of thickness equal to $2l$ [17]. In this equation M_t is the water uptake at time t, M_∞ is the water uptake at equilibrium and D is the average diffusion coefficient of water in the membrane.

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left[- \frac{D(2n+1)^2 \pi^2 t}{l^2} \right] \qquad \textbf{(3)}$$

The experimental points were fitted by means of a non-linear least squares procedure for the first sixteen terms of the sum in equation (3). In this procedure the parameter P_1, defined as D/l^2, was optimized. The data were satisfactorily adjusted to equation (3), as shown in Figure 1.

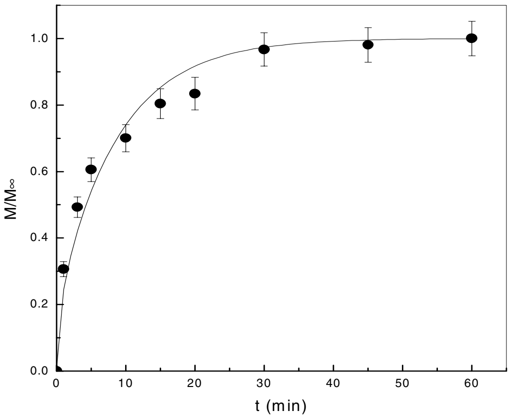
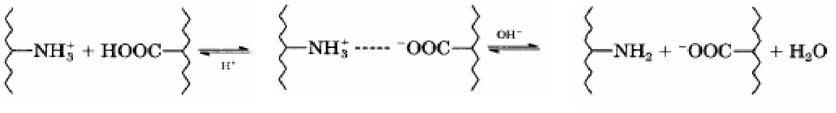


Figure 1. Swelling in water of the PEC membrane. The dots are the experimental points and the curve is the resulting fit of the data to equation (3).

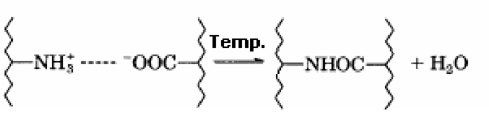
Influence of the cross-linking process on the swelling degree of the PEC membranes

During the swelling process the integrity of the PEC network is maintained by the cross-linking of chains produced by the inter-chain $-NH_3^+\,^-OOC-$ salt bonds. However, at low or high pH these bonds can be broken, resulting in the disintegration of the PEC and dissolution of the membrane, as represented in the following equilibrium:



(4)

This pH instability can be modified by heating PEC membranes at 120ºC under nitrogen atmosphere. This way covalent amide bonds are formed from the salt bonds [18, 19], as represented in the following scheme:



(5)

The membrane becomes now insoluble whatever the solution pH. Moreover, the degree of swelling at a given pH decreases as result of reaction (5). The extent of amide bond formation for a given membrane depends on the heating time. Therefore this treatment can be used in order to regulate the swelling behavior of these PEC membranes. It can be appreciated in Figure 2 that as the heating time of the membranes increases the maximum swelling degree in water decreases. Larger differences were observed in the swelling degree of the membranes treated during 60 and 120 minutes. This is in agreement with the expected increase in amide type cross-links with the increase in reaction time.

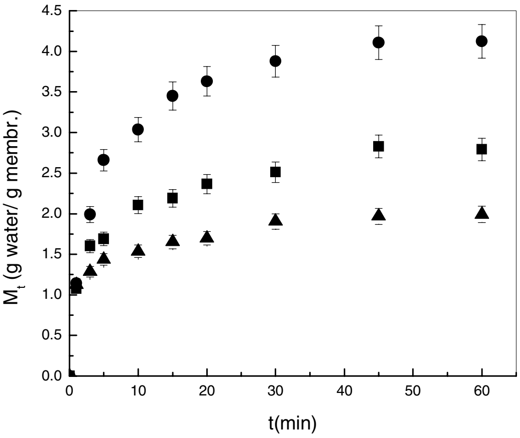


Figure 2. Swelling in water of PEC membranes with different times of heating at 120ºC. (●) original membrane (■) heated 60 minutes (▲) heated 120 minutes.

The swelling process of thermally cross-linked membranes was satisfactorily adjusted to the mathematical solution of the Fick's differential equation. The results are shown in Table 1. The M_∞ values reported correspond to the water uptake achieved after 12 hours. No appreciable variation is observed on the parameter P_1 ($=D/l^2$) resulting from fitting. This almost constancy of P_1 indicates that the expected decrease in D with increasing cross-linking is balanced by the smaller average thickness (l) of the more cross-linked membranes in the swelling state.

Table 1. Maximum equilibrium swelling in water and diffusion coefficient for thermally treated membranes at different times.

Time (min.)	M_∞ (g water per g membrane)	$P_1 = \frac{D}{l^2}$
0	3.84 ± 0.19	0.05± 0.03
30	3.01 ± 0.15	0.08± 0.01
60	2.80 ± 0.14	0.09± 0.01
120	1.99 ± 0.10	0.09± 0.02

Thermal cross-linking process of the complex membranes was also verified by registering the FTIR spectra of the thermally treated membranes. The FTIR spectra of three PEC membranes are shown in Figure 3. The absorption band at 1744 cm^{-1} corresponds to the carbonyl bonds of carboxylate groups of pectin while the absorption bands at 1154 cm^{-1} (anti-symmetric stretching of the C-O-C bridge), 1082 and 1032 cm^{-1} (skeletal vibrations involving the C-O stretching) are characteristic of the saccharide structure of both macromolecular components of the membranes. It can be appreciated that the intensity of the band at 1744 cm^{-1} decreases with the reaction time as a consequence of the formation of amide bonds.

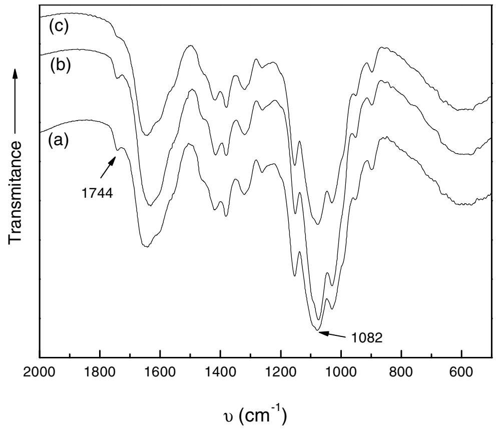


Figure 3. Infrared spectra of PEC membranes with different heating times. (a) original membrane (b) heated 60 minutes (c) heated 120 minutes.

While the absorption band at 1744 cm^{-1}, A_{1744}, decreases with increasing heating time, the intensity of the absorption band at 1082 cm^{-1} should remain invariant. Therefore, the absorbance ratio A_{1744}/A_{1082} can be used to assess the extent of amide bond formation. It can be observed in Figure 4 that the decrease in the ratio A_{1744}/A_{1082} resulting from the increase in heating time is in excellent agreement with the corresponding decrease in M_∞.

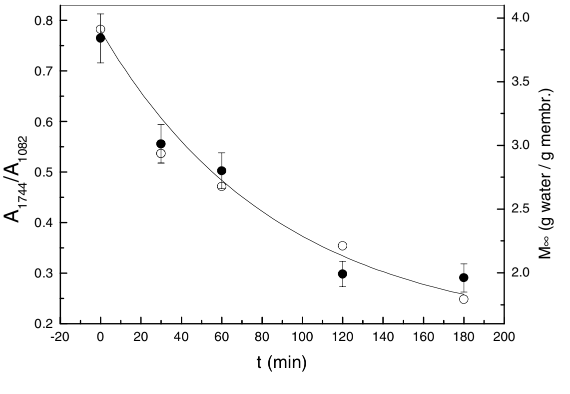


Figure 4. Time variation of the absorbance ratio (○) and maximum swelling in water (●) of PEC membranes with the time of heating at 120ºC.

Morphology the PEC membranes

Figure 5 shows the SEM micrographs of the chitosan-pectin PEC membranes after thermal treatment. The micrographs reveal the rough surface of the films, which is typical of PEC membranes obtained by the precipitation method used in the present work, in contrast with the smooth surfaces generally observed when films are obtained by casting and evaporating from homogenous solutions. It is also apparent that the surface morphology of films was not distinguishably altered by the thermal heating at 120°C. This result confirms that swelling of thermally cross-linked membranes is not apparently affected by changes in morphology, but by the more compact structure of the polymer network.

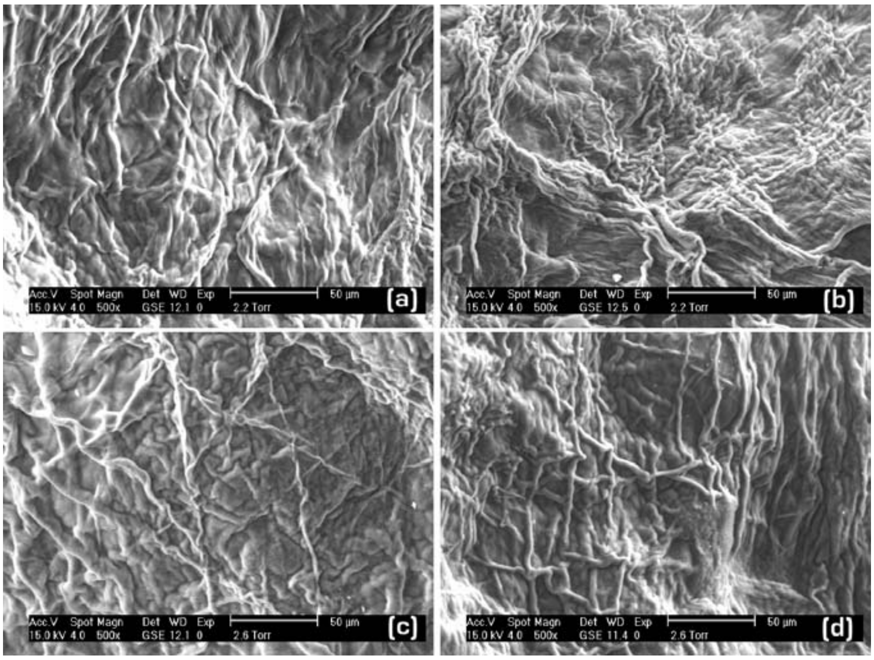


Figure 5. SEM micrographs of PEC membranes after heating at different times. (a) 30 min; (b) 60 min; (c) 120 min; (d) 180 min.

Swelling of the thermally cross-linked PEC membranes

The cross-linked PEC membranes display the expected dependence of swelling on the pH of the solution, as it can be seen in Figure 6. The smaller water uptake is observed at pH 7.4. At pH 12, equilibrium (4) is displaced to the right decreasing the number of ionic cross-links of the PEC network with the consequent increase in swelling. In a similar fashion, at pH 1.2 the equilibrium is shifted to the left resulting also in increased swelling. A similar behavior has been reported for a chitosan/poly(lactic-co-citric)acid hydrogel [20].

It is worth recalling that uncross-linked membranes are totally destroyed at these two extreme pH values. The swelling curves in Figure 5 exhibited Fickean behavior up to $M_t/M_\infty \leq 0.8$.

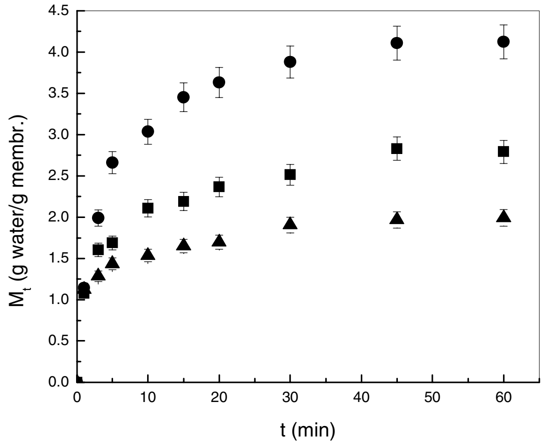


Figure 6. Swelling of Chitosan/Pectin membranes after heating at 120ºC for 60 min., at different pH values. (●) pH 1.2; (▲) pH 7.4; (■) pH 12.

Conclusions

Membranes of the polyelectrolyte complex between chitosan and pectin prepared by a precipitation method are hydrophilic and swell in water. The swelling process is pH dependent and follows Fickean diffusion kinetics. The membranes are destroyed at low and high pH. Thermal heating of the membranes at 120ºC under nitrogen atmosphere brings about the conversion of $-NH_3^+ \ ^-OOC-$ salt bonds into amide bonds, making the membrane insoluble at acid and basic pHs. The extent of the reaction can be monitored by FTIR spectroscopy using the absorbance ratio A_{1744}/A_{1082}. As the reaction time increased, both the absorbance ratio and the maximum swelling of the membrane decreased.

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