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Interpolymer complexes of chitosan and polymethacrylic derivatives of salicylic acid: preparation, characterization and modification by thermal treatment

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Macromolecular interpolymer complexes were prepared by blending concentrated equimolar solutions of chitosan and poly(4-N-methacrylamidobenzoic acid) and evaporation of the solvents. The products obtained presented the structure of interpolymer complexes as indicated by FTIR spectroscopy. The thermal treatment of the solid products at 120°C gives rise to a partial dehydration together with the formation of covalent amide bonds between both polymeric components. The sorption behaviour of the systems is analysed on the basis of the structure of the macromolecular systems and a consideration of Fickian behaviour for highly hydrophilic materials. The diffusion coefficients determined are dependent on the thermal treatment applied to the interpolymer complexes. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

Chitosan is a linear natural polysaccharide composed of a partially deacetylated material of chitin [(1-4)-2-acetamido-2-deoxy-β-D-glucan]. Apart from its biodegradable character in physiological conditions, it is a basic polymer, having reactive amine side groups which offers enormous possibilities of modification, graft reactions and ionic interactions[1–3]. As a polycationic polymer, chitosan has been used for complexation with acidic proteins present in a great variety of biological fluids, owing to the tendency to form polyelectrolyte complexes which can be manipulated easily[4,5]. In addition, chitosan is a highly biocompatible polymeric material with a lethal dose, LD_{50}, as high as 16 g kg^{-1} in mice after oral and intravenous administration[6].

Interpolymer complexes are formed by the association of various macromolecules[7,8] and can be grouped into four major categories depending on the dominant type of interaction.

(1) Stereocomplexes formed by interaction through van der Waals forces.
(2) Polyelectrolytes (or polymeric) complexes which are formed by interactions between macromolecular polyacids and polybases or their salts and are stabilized by ionic bonds.
(3) Complexes formed by hydrogen bonding.
(4) Coordination complexes.

There are complexes formed by a combination of these factors, which in addition give rise to the formation of covalent bonds after the appropriate thermal treatment. In this sense, chitosan is one of the most characteristic polysaccharides which combines with carboxylic and polycarboxylic acids to form intermolecular complexes, giving rise to the corresponding amide functional groups after a single thermal treatment at moderated temperatures[9–12].

The complexation ability of salicylic acid derivatives is well documented in the literature[13,14]. The arrangement of the −OH and −COOH functional groups of the aromatic ring (*Scheme 1*) provides the adequate geometry and stereochemical configuration for the formation of intermolecular complexes with polyvalent cationic ligands. In this sense, we were interested in the study of the complexation process of acrylic derivatives of salicylic acid with pharmacological activity, and chitosan as a highly hydrophilic matrix, as well as the results of thermal treatments of the complexes formed. The present work deals with the preparation, analysis and characterization of interpolymeric complexes between poly(4-*N*-methacrylamidobenzoic acid) and chitosan in solution. In addition, the formation of covalent amide groups after thermal treatment of the complexed systems is discussed on the basis of the analysis of the products obtained.

EXPERIMENTAL

Materials

Chitosan (Deacetylation Degree, D.D. = 79.9% determined by ^{1}H n.m.r., $M_v = 2 \times 10^5$) was obtained from shells of lobsters (*Panulirus argus*) as described elsewhere[16]. Chitosan flakes were dissolved in aqueous acetic acid, filtered and precipitated with aqueous NaOH. The precipitated gel was washed several times with water and vacuum dried in a desiccator. The white chitosan foam thus obtained was stored in a closed flask until used.

Poly(4-*N*-methacrylamidobenzoic acid), P42, was prepared by free radical polymerization of the corresponding methacrylic derivative, in DMF solution at 50°C, initiated by AIBN at high vacuum. The synthesis and polymerization

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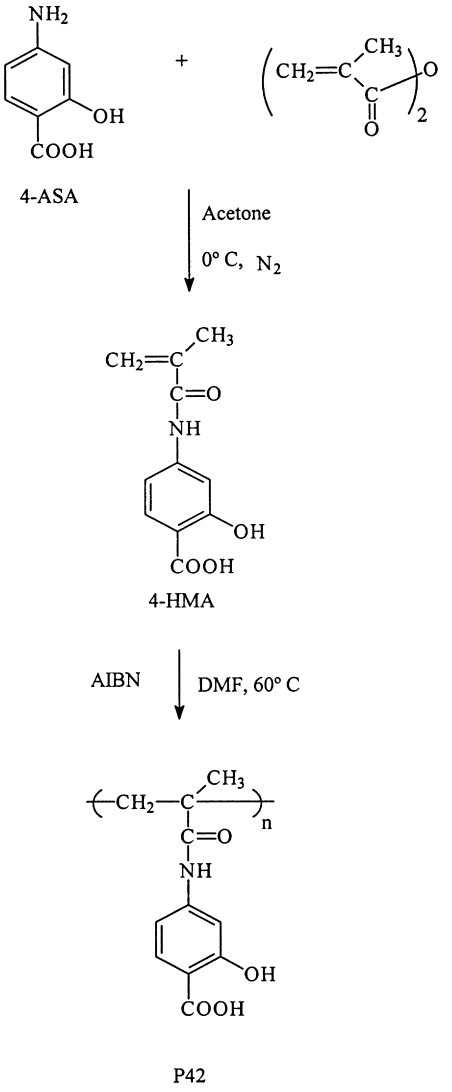

Scheme 1

of the acrylic monomer 4-*N*-methacrylamidobenzoic acid is described in *Scheme 1*. More experimental details are given elsewhere[15]. A P42 of $\bar{M}n = 1.55 \times 10^5$ was used for the preparation of interpolymer complexes with chitosan.

Preparation of polymer blends

Chitosan foam (0.5 g) was swollen in 5 ml of a 45:55 (v/v) dioxan/water mixture. The required equimolecular amount of P42 was dissolved in 8 ml of the same solvent mixture and both systems were left overnight in separate flasks. Then, P42 solution was added to the chitosan gel with gentle manual stirring and the mixture was left to stand for five days. Afterwards it was freeze dried. The polymer blend thus obtained was grounded to a powder and kept in a closed flask until used.

IR spectra

FTIR spectra were recorded on a Nicolet 520 spectrometer. Spectra were taken with a resolution of 2 cm^{-1} and were averaged over 120 scans. Samples were thoroughly ground with exhaustively dried KBr and pellets were prepared by compression under vacuum.

Thermal treatment

Samples were thermally heated in a DSC-4 Perkin Elmer system coupled to a microprocessor System-4 as the temperature control unit. Thermal treatment was performed with 5–6 mg samples on a platinum pan under nitrogen atmosphere with a nominal gas flow rate of 5 ml s^{-1}. Samples were conditioned for 15 min at 30°C and then heated at 80°C min^{-1} until reaching 120°C, taking as zero time the moment at which the system temperature was stabilized automatically.

Sorption experiments

In order to perform sorption studies, disks were prepared by powdering the corresponding polymer blends and compressing them (up to 8 tons) under vacuum on a typical mould for KBr FTIR pellets preparation. The disks were approximately 0.5 mm thick and weighed about 0.1 g. The thickness of each disk was determined as the average of 10 measurements taken with a Militest 1085 (Mahr).

The dry disks were weighed accurately and placed in a closed chamber saturated with water vapour at 37°C. The water uptake, *W*, was calculated by measuring the weight gain of the sample at different times. It was reported as

$$(\%)W = \frac{M - M_0}{M_0} \times 100 = \left(\frac{M}{M_0} - 1\right) \times 100 \qquad (1)$$

where M_0 is the weight of the dry sample and M is that of the sample at time t.

RESULTS AND DISCUSSION

Chitosan is a natural partially deacetylated N-acetyl-glucosamine polysaccharide soluble in dilute aqueous solutions of weak organic acids, e.g. acetic or formic acid as well as in aqueous HCl, while P42 is not soluble in water unless it is in basic medium. Therefore, it is not possible to simultaneously have both polymers together in solution. In order to study the interpolymer reaction of chitosan and P42, a chitosan foam was swelled in a 45% (v/v) dioxan/water mixture, since P42 is soluble in this solvent mixture. Then, to the swelled chitosan gel a solution of P42 with the appropriate composition was added and the mixture left to stand for various days in order to reach equilibrium. Then it was freeze dried and ground to a powder.

With this procedure, blends of chitosan and P42 of three different compositions were prepared. Samples identified as M1, M2 and M3 correspond to a P42 content of 30, 20 and 10 mol%, respectively.

Infrared analysis of chitosan, P42 and the blends

The IR spectra of chitosan, P42 and M1 are shown in *Figure 1*. The chitosan spectrum shows the characteristic absorption bands at 1662 (Amide I), 1600 (-NH_2 bending) and 1380 cm^{-1} (–CH_2 bending). The absorption bands at 1160 cm^{-1} (anti-symmetric stretching of the C–O–C bridge), 1075 and 1040 cm^{-1} (skeletal vibrations involving the C–O stretching) are characteristics of its saccharide structure[17]. The bands at 1672 and 1623 cm^{-1} present in the IR spectrum of P42 are assigned to the carbonyl stretching vibration of acid and amide moieties, respectively, while the strong absorption band at 1596 cm^{-1} with a shoulder at 1552, and the absorption band at 1506 cm^{-1} result from its aromatic character.

The main absorption bands appearing in chitosan and P42 IR spectra are present in the IR spectra of M1, M2 and M3. The fact that the main contribution to the absorption band in the spectral region 1549–1469 cm^{-1} (A1) is due to the aromatic ring from the P42 structure, while the absorption

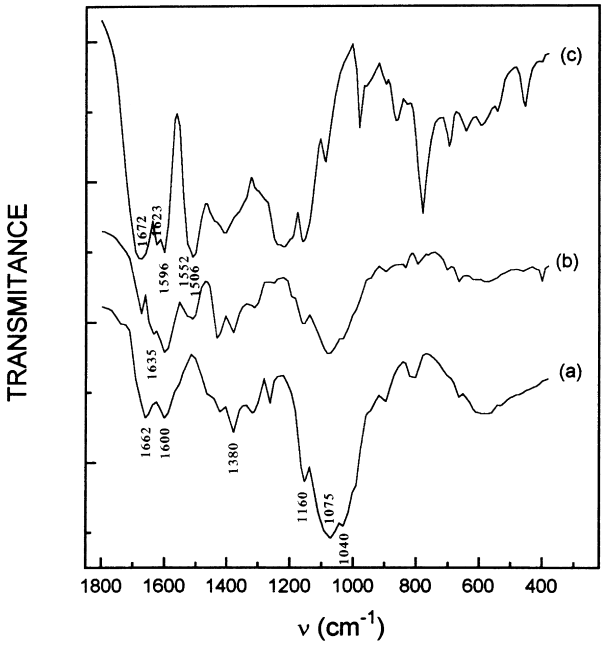

Figure 1 Infrared spectra in the region 400–1800 cm^{-1} for chitosan (a), M1 (b) and P42 (c)

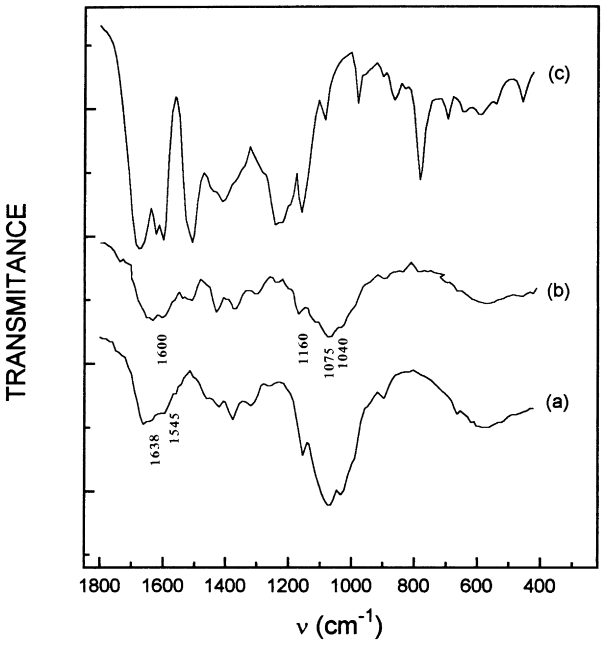

Figure 3 Infrared spectra in the region 400–1800 cm^{-1} for chitosan (a), M1 (b) and P42 (c) after heating at 120°C for 120 min under nitrogen

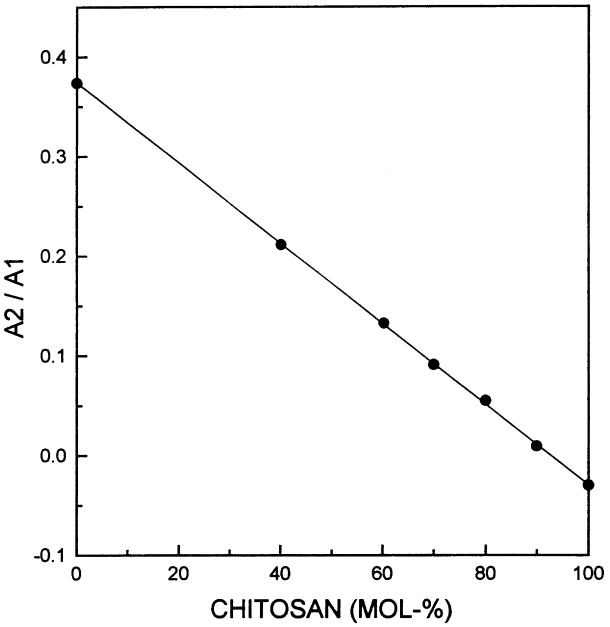

Figure 2 Composition dependence of the ratio of the integrated absorption in the region 1229–915 cm^{-1} (A2) to the integrated absorption in the region 1549–1469 cm^{-1} (A1) for the samples under study

band in the region 1229–915 cm^{-1} (A2) comes mostly from the saccharide structure of chitosan, allows us to obtain an excellent linear correlation (corr. coeff., $r = 0.99992$) between the ratio of the integrated absorption bands, A2/A1, and composition, as shown in *Figure 2*.

The main difference observed in the IR spectrum of M1 as compared with those of chitosan and P42 is the distinctive peak at 1635 cm^{-1} which is not observed in the starting polymers or even in their physical mixture. This is indicative of the existence of a specific interaction between chitosan and P42, most probably through hydrogen bonding between the amino group of the former and the carboxylic OH group of the latter.

The existence of such an interaction is confirmed by subjecting the samples to thermal treatment. The formation of amide chemical links in complexes between polyacids and polyamines upon heating at approximately 120°C has been previously reported[18]. Chitosan citrate films heated in vacuum at 100°C also suffer dehydration with formation of the corresponding amide linkage[19]. In our study, chitosan, P42 and the blends were heated for 120 min at 120°C under nitrogen in a calorimeter. The IR spectra of chitosan, P42 and M1 after this treatment are shown in *Figure 3*.

It can be observed that under this relatively mild heating program, P42 does not suffer any appreciable degradation. In contrast, chitosan shows some signs of degradation, which indicates the appearance of an absorption band at 1638 cm^{-1} with a shoulder at 1545 cm^{-1}, evidencing unsaturation[17]. However, it is worth pointing out that thermal degradation studies on chitosan have shown that at temperatures below 190°C under nitrogen only minor degradation occurs. The first stage of pyrolysis of chitosan in nitrogen starts above 220°C when dehydration, depolymerization and decomposition of the acetylated and deacetylated units of the polymer occur. In this connection it has been shown that the acetylated form is thermally less stable and starts to decompose at a lower temperature than the deacetylated one[17,20].

The intensities of the carbohydrate bands relative to the intensity of the absorption band at 1660 cm^{-1} (resulting mainly from the contribution of the Amide I absorption band) A_{1160}/A_{1660}, A_{1075}/A_{1660} and A_{1040}/A_{1660} decrease with the heating treatment for chitosan as well as for M1. This is to be expected, since the carbohydrate moieties of chitosan have already suffered some degradation. On the other side, while the relative intensity ratios of the carbohydrate absorption bands to that of the absorption band at 1600 cm^{-1} (resulting mainly from the contributions of the aromatic ring of P42 and the amino group of chitosan) A_{1160}/A_{1600}, A_{1075}/A_{1600} and A_{1040}/A_{1600} decreases with treatment, as it should, for chitosan, it increases with treatment for M1. Since the aromatic ring does not suffer any degradation, it

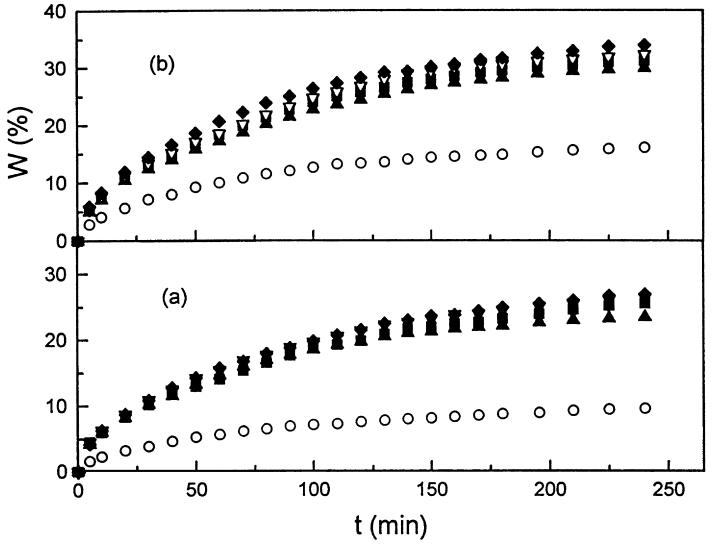

Figure 4 Water vapour sorption isotherms for chitosan (■), P42 (○), M1 (▲), M2 (▼) and M3 (◆) at 37°C: (a) before and (b) after thermal treatment

Table 1 Values of the equilibrium water content, W_∞, and diffusion constant, D, at 37°C for thermally treated and untreated chitosan, P42 and blends

Sample	W_∞ (%)		$D \times 10^9$ ($cm^2\ s^{-1}$)		ΔP (%)
	Untreated	Treated	Untreated	Treated	
Chitosan	87.7	66.9	4.55	9.23	8.5
M3 (10% P42)	88.5	81.1	5.50	8.86	7.6
M2 (20% P42)	80.7	75.8	6.59	9.40	8.4
M1 (30% P42)	71.9	69.4	7.64	10.0	8.1
P42	15.1	22.7	2.36	27.8	6.2

ΔP (%) is the percentage weight loss with treatment.

can be concluded that the increase observed for M1 must be due to the transformation of the amine groups involved in hydrogen bonding and the formation of interpolymer covalent amide links.

Sorption experiments

Figure 4a shows the variation of the water uptake (W) as a function of time for chitosan, P42 and the blends with the three compositions studied, at 37°C. It can be observed that there is an appreciable difference in water sorption ability between chitosan and P42. The water uptake of the blends is somewhat higher than that of chitosan, but is not strongly dependent on composition, which is reflected in the equilibrium water uptake, W_∞, obtained after 24 h of treatment, reported in *Table 1*. The great difference in the limiting water uptake of chitosan and P42 becomes apparent, and it can be seen that W_∞ decreases with increasing proportion of P42 in the blend.

When the rate-determining step in a sorption process is the diffusion of solvent into the swollen matrix, there is a linear dependence between the solvent uptake and $t^{1/2}$, and the system is said to exhibit Fickian behaviour. On the contrary, if the advancement of the swollen–unswollen boundary is slower than the diffusion of the solvent in the swollen polymer, zero order kinetic behaviour is observed and the water uptake increases linearly with the swelling time[21].

The water uptake *versus* $t^{1/2}$ diagrams obtained at 37°C for chitosan, P42 and the blends are shown in *Figure 5a*. It can be appreciated that Fickian behaviour is obeyed up to approximately 100 min of swelling, although for longer times the behaviour deviates from linearity.

The solution of the differential form of Fick's second law for thin sheets neglecting diffusion through the edges can be expressed as a function of the reduced uptake M_t/M_∞ (or W_t/W_∞) and $t^{1/2}$ according to the following relationship[22]:

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{Dt}{\pi l^2}} = \frac{W_t}{W_\infty} \qquad (2)$$

Here, M_t and M_∞ represent the water uptake at time t and at infinite time, respectively, D is the diffusion coefficient and l is the average thickness of the disk.

From the straight lines of the W_t *versus* $t^{1/2}$ plots, and taking into consideration the value of W_∞ reported in *Table 1* and the thickness of the disk, the diffusion coefficients of samples were determined. The values obtained are reported in *Table 1* and they show a clear dependence of D with composition.

For prolonged swelling times, the experimental points seem to deviate from Fickian behaviour, characterized by the straight lines of the W *versus* $t^{1/2}$ plots. However, as shown in *Figure 6a*, they adequately fit the expression proposed by Schott[23] for the extensive swelling of polymers in the form:

$$\frac{t}{W} = A + Bt \qquad (3)$$

where W is the water uptake at time t, $B = 1/W_\infty$, the inverse of the maximum swelling, and $A = 1/(dW/dt)_0$, the reciprocal of the initial swelling rate. In fact, Schott demonstrated that equation (3) implies second order swelling kinetics.

The diagrams obtained by the application of equation (3) to the data in *Figure 4* for long swelling times give straight

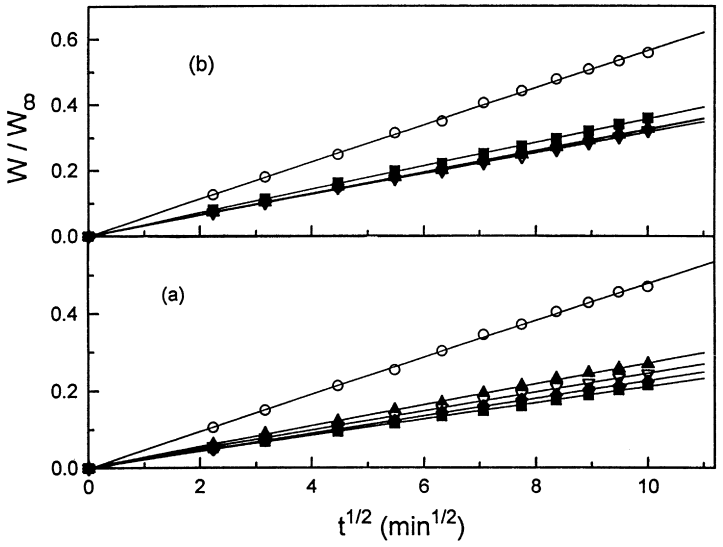

Figure 5 Water uptake as a function of $t^{1/2}$ for chitosan (■), P42 (●), M1 (▲), M2 (▼) and M3 (♦) at 37°C: (a) before and (b) after thermal treatment

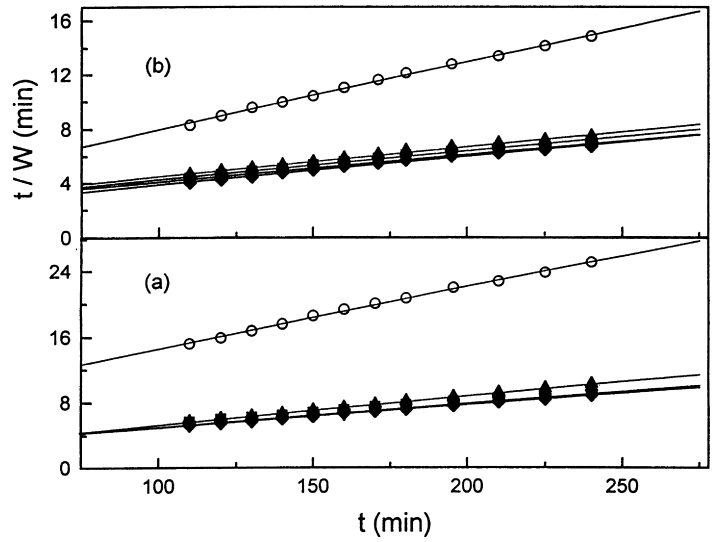

Figure 6 Reciprocal of the average swelling rate, t/W, as a function of the time of treatment for chitosan (■), P42 (●), M1 (▲), M2 (▼) and M3 (♦) at 37°C: (a) before and (b) after thermal treatment. Straight lines correspond to the least squares fit of experimental points to equation (3)

lines with excellent correlation coefficients, as is illustrated in *Figure 6a* for the swelling data of the five samples.

In order to study the effect of thermal treatment on the sorption behaviour of samples, disks of chitosan, P42 and the blends were heated for 120 min at 120°C under nitrogen in an oven. Water uptake curves of thermally treated chitosan, P42 and blends are shown in *Figure 4b*. The general pattern is very similar to that of unreacted samples in that chitosan seems to control sorption. The values of the limiting uptake for all samples are listed in *Table 1*. There is a very significant increase in the limiting sorption of P42 with treatment, but if one considers the water lost during the heating process (6.2 wt%) it can be readily shown that the final hydration degree of treated P42 is the same as that of the untreated sample on a dry polymer basis. This is not surprising since, as was shown from IR analysis, the polymer does not suffer noticeable alterations of the chemical structure by this thermal treatment.

On the other side, W_∞ decreases with treatment for chitosan and the blends. In the case of chitosan, the smaller value obtained for W_∞ must be related to the certain dehydration produced during the heating program. The decrease in the limiting uptake of the blends with thermal treatment is more marked in the blends richer in chitosan. This could be the result of the formation of amide links during heating and some dehydration of the polysaccharide units. It must be recognized, however, that the magnitude of the effect is not very high.

As with the untreated samples, Fickian behaviour is obtained for the first 100 min of sorption, as depicted in *Figure 5b*, while linear Schott diagrams are obtained for longer times, as shown in *Figure 6b*. From the slope of the lines in the W_t/W_∞ *versus* $t^{1/2}$ plots, the diffusion coefficients were evaluated as before. They are listed in *Table 1*. In all cases, the D values of the treated samples are higher than those of the corresponding untreated ones and also increase with increasing P42 content.

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