Macromol. Symp. **2001,** *168, 103-116*

Chitosan Based Polyelectrolyte Complexes

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SUMMARY: Polyelectrolyte complexes (PECs) are formed through the electrostatic interactions between polymers carrying opposite charges. Here are presented results of basic studies on the PECs of chitosan with other polysaccharides such as sodium alginate, carboxymethyl cellulose, polygalacturonic acid and κ-carrageenan. An extensive study on chitosan/carboxymethyl cellulose membranes, regarding its swelling characteristics and water vapour sorption is offered. Also the interaction of chitosan with polyacrylic acid has been examined from the thermodynamic point of view.

Introduction

Polyelectrolyte complexes of chitosan (CHI) with other polysaccharides are usually found in biotechnological and pharmaceutical applications. Microcapsules of CHI and sodium alginate or carboxymethyl cellulose (CMC) have been prepared for encapsulating cells, proteins and enzymes. The capsular wall consists of a membrane of the PEC whose characteristics govern properties that are usually decisive for their end use, such as: biocompatibility, biodegradability, burst strength and permeability. Complexation of CHI with other polyacids has been used for sustained drug release formulations. However, in spite of the increasing number of applications of these complexes there is still a lack of fundamental work in order to deep into the knowledge about the influence of many of the factors affecting the extent of the polymer-polymer complexation and the properties of the membranes prepared with them.

We have studied the polyelectrolyte complexes of chitosan with other polysaccharides: sodium alginate, CMC, polygalacturonic acid and κ-carrageenan. An extensive study on CHI/CMC membranes, regarding its swelling characteristics and water vapour sorption was performed. Also the interaction of chitosan with PAA has been examined from the thermodynamic point of view. Some of our findings are presented here.

CCC 1022-1360/00/$ 17.50+.50/0

Interaction of chitosan with polycarboxylic acids

The interaction of chitosan (CHI) with carboxylic acids leads to the formation of an insoluble polyelectrolyte complex[1-3] . Chitosan is insoluble in neutral and basic media, therefore the PEC is usually obtained by mixing equimolar quantities of the polyacid and chitosan hydrochloride. The complex is formed according to the following reaction

$$\begin{array}{ccccccc} \sim\!\sim COOH & + & {}^{+}NH_3\!\sim\!\sim & \rightleftharpoons & \sim\!\sim COO^{-}\,{}^{+}NH_3\!\sim\!\sim & + & H^{+} \\ C_0(1-\theta) & & C_0(1-\theta) & & C_0\theta & & C_0\theta \end{array} \qquad (I)$$

As a result, the pH of the solution decreases. The degree of conversion of the reaction, θ, expressed as the ratio of the concentration of interchain salt bonds formed, C_k, to the initial concentration of any of the polyelectrolytes, C_0, can be evaluated from pH measurements by:

$$\theta = ([H^+] - [H^+]_{PA})/C_0 \qquad (1)$$

where $[H^+]$ and $[H^+]_{PA}$ are the concentration of hydrogen ions in the reaction mixture and in a solution of the polyacid at the same concentration, respectively. A value of $\theta = 0.10$ and 0.13 was obtained at 25°C when the polyanions were CMC and PAA, respectively, indicating a very low degree of conversion of the reaction[3]. However, the equilibrium reaction (I) can be shifted to the right by addition of a strong base. In so doing θ can be assessed from the data of the potentiometric titration of the mixture[4].

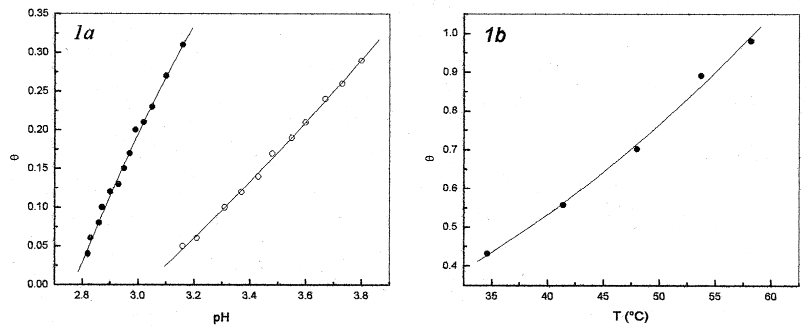

Fig. 1: *a*) Dependence of θ on pH (●) CHI/CMC (O) CHI/PAA polyelectrolyte complexes.
b) Temperature dependence of θ for the CHI/CMC system.

The dependence of θ on pH is shown in Figure 1*a* for the systems CHI/CMC and CHI/PAA. The high slopes of the curves are typical of cooperative transitions and the difference between them must be related to the different structure and pK_o values of the polyacids. As it can be also seen in Figure 1*b*, temperature has also an important effect on the degree of conversion of the PEC, which increases dramatically from 0.4 at 35°C to almost unity at 60°C for the CHI/CMC polyelectrolyte complex. This abrupt change in θ with temperature must have important consequences on the properties of the PEC, such as the swelling behaviour, since for $\theta = 1$ there would be no free ionic groups in the complex.

The stoichiometry of PECs of weak polyacids and weak polybases is pH dependent, due to the variation of their dissociation degree with pH. The composition of the PEC is given by $\alpha_{PA}[PA] = \alpha_{PB}[PB]$ where [PA] and [PB] are the molar compositions of the polyacid and the polybase in the PEC and α_{PA} and α_{PB} are their respective dissociation degrees[5].

Due to the insolubility of the PEC its accumulation can be followed by turbidimetric methods. The value of Z at maximum turbidity gives the stoichiometry of the PEC. It has been reported that when CHI solution is added to a solution of CMC at pH 3.6 the composition of the PEC is rich in the polyacid, while at pH 4.8 the excess component is CHI. At pH 4 the maximum turbidity is located at Z ≅ 1 indicating a 1:1 stoichiometry[6].

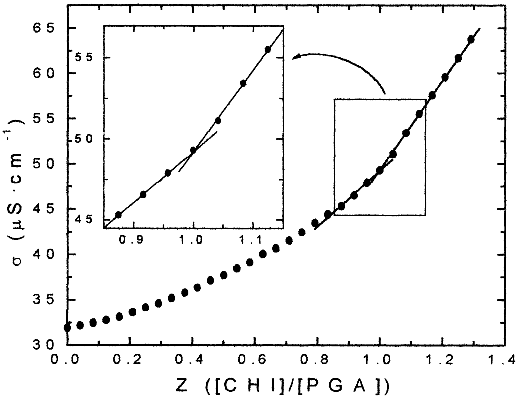

Fig. 2: Conductimetric curve corresponding to the addition of CHI solution (4.59×10^{-3} equiv·L^{-1}) to that of NaPGA (4.59×10^{-4} equiv·L^{-1}) as a function of Z([CHI]/[PGA]).

The interpolymer reaction between CHI and sodium polygalacturonate (NaPGA) was followed by conductimetry$^{7)}$. Figure 2 shows the specific conductivity of the reaction mixture as a function of the molar fraction Z (=[CHI]/[PGA]), during PEC formation when CHI·HCl solution is added to a solution of NaPGA. The equivalence point is at Z = 1.

The interpolyelectrolyte reaction can be represented as:

$$\begin{array}{ccccc} P^-Na^+ + P^+Cl^- & \rightleftharpoons & P^-P^+ + Na^+ + Cl^- \\ (1\text{-}Z\theta) \quad Z(1\text{-}\theta) & & Z\theta \quad Z\theta \quad Z\theta \end{array} \qquad \text{(II)}$$

Taking into account the additivity of the contributions of all ionic species, it can be shown that the specific conductivity, $\sigma(Z)$, of the reaction medium is given by:

$$\sigma(Z) = C(1\text{-}Z\theta)P_1 + CZ(1\text{-}\theta)P_2 + CZ\theta P_3 \qquad (2)$$

In the above expression C is the concentration of the polyelectrolyte, expressed in equiv.L^{-1}, P_1, P_2 and P_3 involve the contribution of the different species present in solution to the total conductivity. Thus the contribution to the specific conductivity of NaPGA in solution, represented as P_1, results from the following equilibrium:

$$\begin{array}{ccc} \sim\!\sim\!\sim COO^{-}\,{}^{+}Na + H_2O & \rightleftharpoons & \sim\!\sim\!\sim COOH + OH^- + Na^+ \\ \alpha & & 1\text{-}\alpha \quad 1\text{-}\alpha \quad 1\text{-}\alpha \end{array} \qquad \text{(III)}$$

P_1 can be calculated using the following expression:

$$\sigma_{PGA}/C = P_1 = (1\text{-}\alpha)(\lambda_{Na+} + \lambda_{OH-}) + \alpha f_{PGA}(\lambda_{P-} + \lambda_{Na+}) \qquad (3)$$

where λ_i is the equivalent ionic conductivity of species of type *i* and f_{PGA} is the transport coefficient of PGA. λ_{P-} and f_{PGA} are 56.8 x10^{-4} m^2·S.mol^{-1} and 0.64, respectively$^{7)}$.

Similarly, in order to evaluate the contribution of chitosan hydrochloride to the conductivity of the solution, P_2 (= σ_{CHI}/C), its dissociation equilibrium

$$\begin{array}{ccc} \sim\!\sim\!\sim NH_3^{+}\,{}^{-}Cl + H_2O & \rightleftharpoons & \sim\!\sim\!\sim NH_2 + H_3O^+ + Cl^- \\ (1\text{-}\alpha') & & \alpha' \quad \alpha' \end{array} \qquad \text{(IV)}$$

has to be taken into account. Then

$$\sigma_{CHI}/C = P_2 = \alpha' (\lambda_{H+} + \lambda_{Cl-}) + (1-\alpha') f_{CHI}(\lambda_{P+} + \lambda_{Cl-}) \qquad (4)$$

where $\lambda_{P+} = 3.14 \times 10^{-4}$ m^2.S.mol^{-1} and $f_{CHI} = 0.82$[7].

In equation (2), $P_3 = \sigma_{NaCl}/C = (\lambda_{Na+} + \lambda_{Cl-})$ = constant . P_3 can be readily calculated for a given concentration from the ionic conductivities of Na^+ and Cl^- ions

Due to the insolubility of the PEC, it can be assumed that for $Z \leq 1$ all the polyelectrolyte incorporated in it do not contribute to the conductivity of the reaction mixture. Then

$$\theta = [\sigma(Z) - C(1-Z)P_1]/CZP_3 \qquad (5)$$

P_1 can be evaluated experimentally from the slope of the dependence of σ_{PGA} on PGA concentration when a solution of PGA is added to a NaCl solution of the same concentration as that of CHI solution used in the interpolymer reaction.

In Fig. 3 the values of the degree of complexation estimated with equation (5) are plotted as a function of Z. It is evident that when the first few amounts of chitosan are added to the reaction system the functional groups of both polyelectrolytes become completely complexed, with θ values falling very rapidly to nearly 0.80, possibly due to steric reasons. At higher values of molar composition of the mixture there is a slight increment in the value of this parameter raising up to 0.85 for Z = 1.

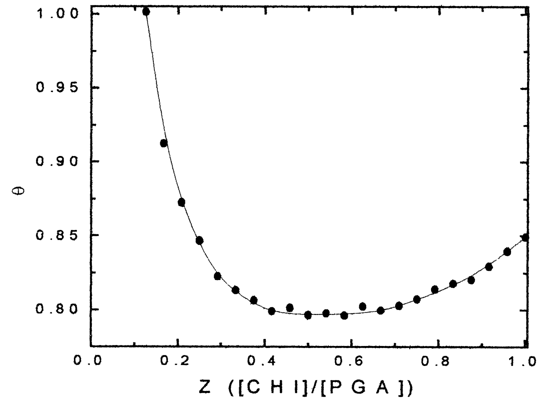

Fig. 3: Values of θ calculated by equation (5) as a function of Z([CHI]/[PGA]).

PECs of CHI and alginate (ALG) are used in microcapsules designed for biotechnological purposes. Alginic acid is essentially a linear copolymer composed of mannuronic (M) and guluronic acid (G) units. Chemical composition and molecular weight of commercial ALGs are conditioned by their source and is quite variable. The same occurs with chitosan. For that reason, the possible effect of composition and molecular weight of these polysaccharides on the stoichiometry of the PEC was evaluated. The alginates employed had an M/G ratio of 1.96 (ALG-1), 1.31 (ALG-2) and 0.44 (ALG-3). The molecular weights were 7.2 x 10^4, 3.6 x 10^5 and 1.0 x 10^4.

Experiments were carried out titrating sodium alginate solutions (5 x 10^{-4} mol.L^{-1}) with chitosan hydrochloride (5 x 10^{-3} mol.L^{-1}). The interpolyelectrolyte reaction was followed by both conductimetry and potentiometry. A typical outcome is shown in Figure 4. The value of Z (=CHI/ALG) at the end of the titration corresponds to the stoichiometry of the complex. The values of Z obtained by both methods coincide within experimental error. They ranged from 0.67 to 0.75, with no definite trend, indicating that the composition of the complex is independent of the molecular weight of chitosan as well as the composition of the alginates used.

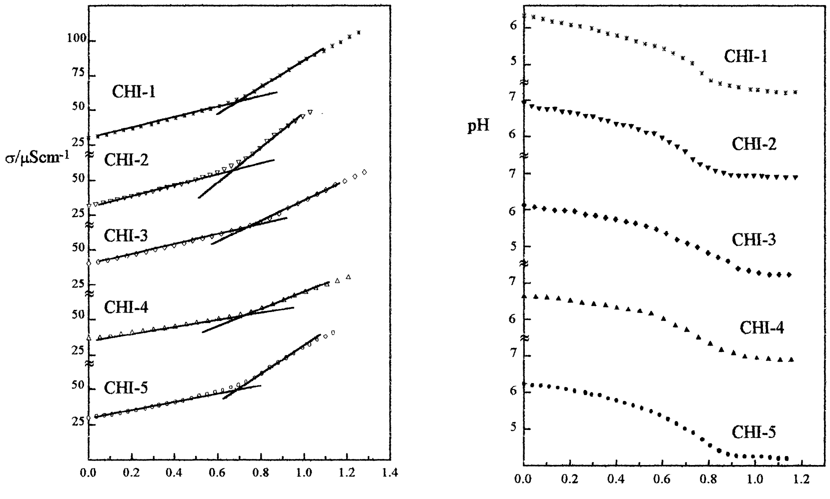

Fig. 4: Conductimetric (left) and potentiometric (right) titrations of sodium alginate solution (ALG-1) with different molecular weight chitosan samples: CHI-1, 23.0 × 10^4; CHI-2, 15.7 × 10^4; CHI-3, 4.6 × 10^4; CHI-4, 1.6 × 10^4; CHI-5, 0.5 × 10^4.

The reason for this becomes apparent when one realises that all alginate samples had about the same pK_0 (3.05 – 3.10) which seems to be the relevant parameter in this case. The determination of the dissociation degrees of both polysaccharides at the end point of the titration (pH 5) for calculating Z (CHI/ALG) predicted a complex composition of 0.73 ± 0.02.

Cooperativity of the interpolymer reaction

The cooperativity of the interpolyelectrolyte reaction between CHI and weak polyacids is well illustrated for the system CHI/PAA[8]. Reaction (I) can be represented as the result of the following reactions:

$$\sim\!\!\sim COOH \xrightleftharpoons{K_d} \sim\!\!\sim COO^- + H^+ \qquad (V)$$

$$\sim\!\!\sim COO^- + {}^+NH_3\sim\!\!\sim \xrightleftharpoons{K_2} \sim\!\!\sim COO^-\,{}^+NH_3\sim\!\!\sim \qquad (VI)$$

The existence of cooperativity implies that the reaction constant for the formation of bond *i*, K_2^i, is bigger than the reaction constant for the formation of bond *i*-1, K_2^{i-1}. It has been shown that the magnitude of this effect can be adequately quantified by expressing K_2 as $K_2 = K_2^0 \cdot \exp^{-m\theta}$, where K_2^0 is the equilibrium constant for the formation of the first interchain bond[9].

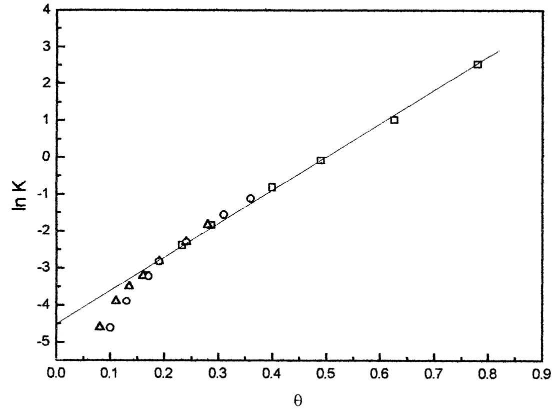

Fig. 5: Relationship between the stability constant (K) and θ for the CHI/PAA complex for different molecular weight chitosans (DA= 20.1%) (□) Mv = 2.3 x 10^5; (o) Mv = 1.1 x 10^5; (Δ) Mv = 0.85 x 10^5. PAA molecular weight: Mv = 2.9 x 10^5.

Figure 5 shows the linear dependence obtained for ln K on θ for the system CHI/PAA in the interval $0.2 < \theta < 0.9$. From the slope of this line, m was evaluated and found to be $m = -9.09$. The large negative value of m indicates a marked increase in K_2 with increasing θ, resulting in the formation of long sequences of consecutive bonds.

Membranes of the CHI/CMC interpolymer complex

The properties of PEC membranes are strongly dependent on the preparation conditions –pH, ionic strength, temperature, concentration and molar ratio of reacting polyelectrolytes, among others[10].

The membranes are rigid and transparent in the dry state, with glass transition temperatures ranging from 115°C for a PEC formed at pH 5.51 (PEC-5) to 125°C for the PEC formed at pH 4.46 (PEC-4)[9]. They swell considerably in water, as it can be appreciated in Figure 6. The contraction experienced by the PEC membranes after maximum swelling can be explained considering two factors: a) the small ions trapped diffuse out of the membrane, decreasing the osmotic pressure and b) at pH 5.5 the free carboxylic groups of the CMC chains are mostly as sodium carboxylate and the amino groups of chitosan are protonated, so that new salt bonds can be formed through the following reaction:

$$\sim\!\sim COO^-Na^+ + Cl^{-+}NH_3\sim\!\sim \;\rightleftharpoons\; \sim\!\sim COO^{-+}NH_3\sim\!\sim + Na^+ + Cl^- \qquad \text{(VII)}$$

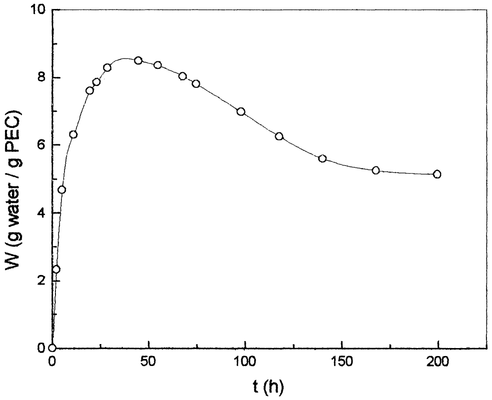

Fig. 6: Typical swelling curve of a CHI/CMC PEC membrane in water at 25°C (pH = 5.5).

The segmental mobility in the swelling state must be sufficient for this reaction to proceed[10]. As a result of this θ raises in time, increasing the retractile force of the polymer network. Reaction (VII) is favoured by the migration of Na^+ and Cl^- ions towards the solution and the cooperativity of the reaction.

When membranes at maximum swelling were placed in solutions containing $CaCl_2$ a volume contraction was observed[12,13]. The magnitude of this effect can be appreciated in Fig. 7 where it is shown in a logarithmic scale the equilibrium swelling, W_∞, reached after contraction as a function of Ca^{2+} concentration for membranes formed at different pH values. This effect was not produced by other salts, such as NaCl, KCl, $NaNO_3$ and Na_2SO_4, indicating the existence of a specific effect of Ca^{2+} ions on the free carboxylate groups of the PEC, possibly by the formation of Ca^{2+} complexes with fixed ligands of the network. This complexation can be visualised as the formation of new crosslinks, which would increase the retractile force of the PEC's macromolecular network, thus provoking its contraction. The effect increases with increasing the pH of formation of the membranes, since at higher pH there exists a greater amount of free carboxylate groups.

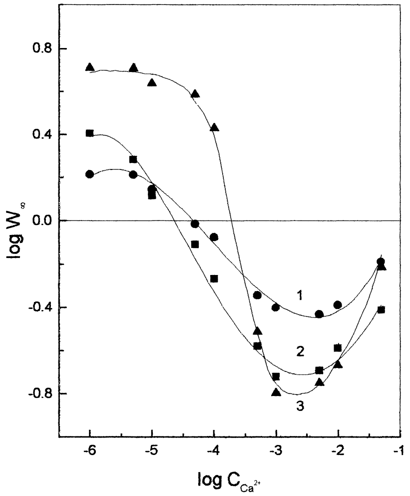

Fig. 7: Logarithmic plot of the final swelling (W_∞) vs. Ca^{2+} concentration for CHI/CMC PEC membranes formed at three different pH values. (1) 4.43; (2) 5.35; (3) 5.70.

The permeability to phenylalanine of CHI/CMC membranes in acid (pH 2) and basic (pH 12) media was followed. Table 1 shows the values of the permeability coefficients, P, obtained for two membranes prepared at different pH (referred to as M1 and M2). Addition of $CaCl_2$ (conc. 0.001 mol.L^{-1}) during one permeability experience in basic medium decreased P to half its value, but no change was observed when addition took place in acid medium. Therefore, the variation of the swelling degree with pH and Ca^{2+} concentration can be utilised to control the velocity of fluxes through the membranes as well as their permselectivity by varying the degree of swelling as a result of changing the environmental conditions.

Table 1. *Permeability to phenylalanine of two CHI/CMC PEC membranes*

Membrane	W* (g H_2O/g PEC)	P_{HCl} (cm^2/s)	P_{NaOH} (cm^2/s)
M1	2.7	2.51×10^{-6}	1.19×10^{-6}
M2	5.0	3.14×10^{-6}	2.08×10^{-6}

**These values correspond to the maximum swelling state in water*

Sorption of water vapour by CHI/CMC PEC membranes prepared at two different pH exhibited a linear dependence of water uptake (W) on time for values of W lower than 0.5g of water per gram of membrane. For higher values of W diffusion becomes controlled by the relaxation of chains, and second-order kinetics is observed (Figure 8). Sorption curves adjusted satisfactorily to the following equation:

$$W /[(W_\infty - W)W_\infty] + KW = kt \tag{6}$$

where k and K are two constants$^{11)}$.

The apparent diffusion coefficients were 3.3×10^{-8} $cm^2 \cdot s^{-1}$ for PEC-5 and 6.3×10^{-8} $cm^2 \cdot s^{-1}$ for PEC-4 at 30°C and water vapour pressure of 23.5 mm Hg.

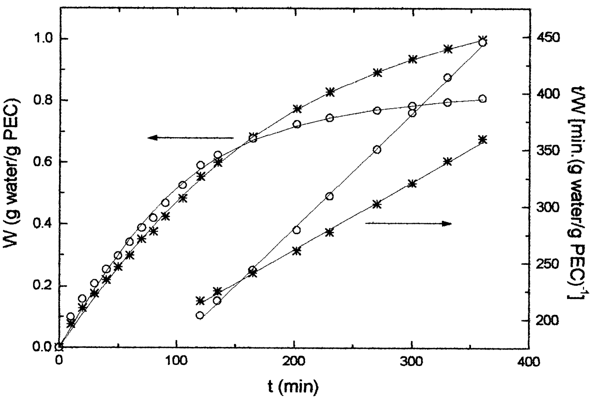

Fig. 8: Dependence of the water uptake (*W*) and the reciprocal of the average sorption rate (*t*/*W*) on time. Points correspond to experimental values: (o) PEC-4; (*) PEC-5. Curves were calculated with equation (6). Straight lines represent the linear regresion of the *t*/*W* vs. *t* plots.

Chitosan/(polyacrylic acid) semi-interpenetrated polymer networks

Polymerisation of acrylic acid in the presence of chitosan produce semi-interpenetrating networks (semi-IPN) of chitosan and PAA. The combination of interpolymer complex and semi-IPN structure leads to novel polymeric materials with special properties, potentially useful in various fields, including controlled drug delivery and dentistry. A self curing CHI/acrylic acid system in physiological conditions with potential applications as biodegradable filling systems and controlled release devises was developed[14].

CHI/PAA semi-IPN membranes were highly hydrophilic, with an equilibrium water content (W_∞) which is highly dependent on pH and composition, as shown in Fig. 9 for experiments carried out at 37°C. The same pattern is observed in experiments conducted at 25 and 50°C. For a given composition, W_∞ increases with increasing pH, due to the breakage of the interpolyelectrolyte salt bonds and the dissociation of the free COOH groups.

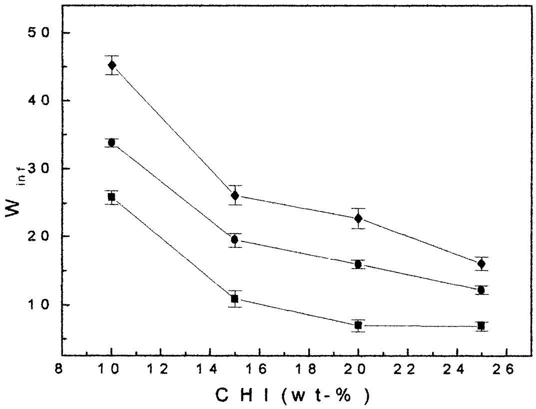

Fig. 9: Equilibrium swelling degree at 37°C of CHI/PAA membranes as function of composition for different pH values. pH 5 (■); pH 7 (●); pH 10 (◆).

Influence of complexation on gelation of κ-carrageenan

Gellation of κ-carrageenan (CAR) takes place as a direct consequence of helical ordering and subsequent aggregation. The process is induced by monovalent cations, both selective and non-selectively, but suppressed under non-aggregating conditions (e.g. in the presence of tetramethyl ammonium salts)[15)].

The influence of complexing very short chitosan chains with DP = 20 referred to as CHI-20, with CAR on its gelling behaviour was studied[16)]. Under the conditions used a non-stoichiometric polyelectrolyte complex (N-PEC) is formed. The gelation of CAR was monitored by strain controlled small deformation oscillatory measurements.

The evolution of the storage modulus at $\omega = 1$ rad·s^{-1} during cooling of a κ-carrageenan solution alone and in combination with CHI-20 in NPECs of varying composition (Z=0.028 to 0.084), at identical CAR concentration in 0.25 mol·L^{-1} NaCl is presented in Fig. 10. Inspection of the individual traces reveals a general elevation of the final G' values of the NPEC at low temperatures as the proportion of complexed CHI increases. Moreover, the onset temperature of gelation (Tg) of the NPECs is shifted to higher temperature, regardless of the NPEC composition. It should be noted that in two of the complexes (Z=0.042 and 0.084) the G' moduli registered at temperatures above Tg are greater than the rest of the complexes and κ-carrageenan alone.

According to these results it could be argued that the complexation of chitosan with κ-carrageenan coils in the presence of 0.25 mol·L^{-1} NaCl leads to a reduction of charge density, due to charge compensation, and hence to the stabilisation of the ordered form of this gelling biopolymer. Besides, the obtained gel network is stronger that that of κ-carrageenan at the same equivalent concentration. This effect is rationalised in terms of the formation and self-association of hydrophobic PEC junction zones17) at temperatures well above that of the sol gel transition (Fig. 10) -leading to increase in G' at high temperature-. The slightly greater mechanical strength can be a consequence of secondary aggregation of such PEC hydrophobic 'patches' bound to be reinforced by the addition of NaCl17-19), a non selective electrolyte for κ-carrageenan20). Hence, such complexed regions can be conceived as microphase separated inclusions in a sort of block copolymer gel network.

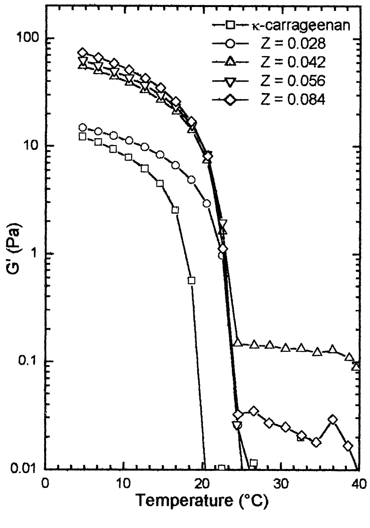

Fig. 10: Temperature dependence of G' (1 rad·s^{-1}; $\gamma = 0.15$; 1°C·min^{-1}) for κ-carrageenan in NaCl alone and in complexes with chitosan (CHI-20) of varying Z (as indicated in label).

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