ELSEVIER

Polymer 41 (2000) 2373–2378

polymer

Conductimetric study of the interpolyelectrolyte reaction between chitosan and polygalacturonic acid

W. Argüelles-Monal[a,*], G. Cabrera[b], C. Peniche[c], M. Rinaudo[d]

[a]*Instituto de Materiales y Reactivos, Universidad de La Habana, Havana 10400, Cuba*
[b]*INCA, San José de las Lajas, Havana, Cuba*
[c]*Centro de Biomateriales, Universidad de La Habana, Havana 10400, Cuba*
[d]*Centre de Recherches sur les Macromolécules Végétales, CNRS, affiliated to the Joseph Fourier University of Grenoble, BP 53X, 38041 Grenoble Cedex 9, France*

Received 27 January 1999; received in revised form 20 May 1999; accepted 1 June 1999

Abstract

The interpolyelectrolyte reaction between chitosan hydrochloride (CHI) and sodium polygalacturonate (NaPGA) has been investigated by conductimetric measurements. The composition of the reaction mixture, Z, was assessed by monitoring the conductivity when a CHI solution was added to a solution of NaPGA. It was determined to be unity at the equivalence point, indicating a 1:1 stoichiometry for the complex. The degree of complexation, θ, expressed as the fraction of groups reacted, was also obtained from the conductimetric measurements by means of a simple procedure. To this end the limiting ionic conductivities and transport coefficients of CHI and NaPGA were previously evaluated. It was found that θ varies with Z. It falls from unity to almost 0.8 as Z increases from 0.2 to 0.5 and then rises again up to 0.85 for $Z = 1$. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Polygalacturonic acid; Polyelectrolyte complex

---

1. Introduction

Polyelectrolyte complexes (PEC) result from the interaction of macromolecules carrying electrostatically complementary ionizable groups. They have been proposed for the design of drug delivery systems, protein separation, anticoagulant coatings, and membranes for separating materials or even as skin substitutes, among other applications [1–3]. The preparation of PECs from natural polymers, such as polysaccharides or polypeptides has the additional advantage of being non-toxic and bioabsorbable [4].

Due to its unique cationic character, chitosan (mainly (1 → 4)-2-amino-2-deoxy-β-D-glucan) has received increasing attention as a polymer component in a variety of such complexes [5–10]. On the other hand, polygalacturonic acid (mainly α-(1 → 4) linked D-galacturonic acid) and pectin have almost been unstudied in the preparation of such complexes [11,12].

Reactions between polyelectrolytes are accompanied by the release to the medium of ionic species with different mobilities converting conductometry in a powerful technique to study these types of processes. Unfortunately, very scarce investigations have been reported on this subject, in spite of the important information that could be obtained [12]. This fact may be justified as it requires careful procedure that must be followed, including meticulous purification of the polymers.

In this paper we report the study of the reaction between two ionic polysaccharides, chitosan hydrochoride and sodium polygalacturonate, using conductometry. The degree of complexation between both oppositely charged ionic groups is estimated as well. It should be noticed that we will restrict our efforts to the case when chitosan hydrochloride is added to the sodium polygalacturonate.

2. Experimental part

2.1. Materials

Chitosan from lobster cephalotorax *Panulirus argus* was prepared industrially in a Cuban enterprise. Its viscosity-average molecular weight was 2.3×10^5 estimated at 25°C in 0.3 M acetic acid/0.2 M sodium acetate [13] and the degree of N-acetylation DA = 0.20 was determined by ^{1}H NMR.

---

[*] Corresponding author.

E-mail address: waldo@imre.oc.uh.cu (W. Argüelles-Monal)

PII: S0032-3861(99)00396-1

Sodium polygalacturonate obtained from orange peels was purchased from Sigma Chemical Co. The characterisation of the chemical composition of this sample gives the following results: galacturonic acid, 87.5%; esterified galacturonic units, 5%; neutral sugars (arabinose, galactose, rhamnose, xylose and glucose), 7.3% as determined by gas chromatography. For this purpose, the sample was previously metanolised, hydrolysed at 120°C with 2 M trifluoroacetic acid and further acetylated. Its viscosity-average molecular weight was 1.02×10^5, as determined by viscometry in 0.1 M NaCl at 25°C [14].

Both polysaccharide samples were carefully purified prior to use. Twice distilled water ($\sigma \approx 3\ \mu\mathrm{S\ cm}^{-1}$) was employed during the experiments. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Purification of chitosan

The polymer ($5\ \mathrm{g\ l}^{-1}$) was dissolved in 1% (v/v) acetic acid and successively filtered through sintered glass filters (pore diameters: 90, 45 and 5 μm) and membranes (0.8 μm). Then it was precipitated by drop-wise addition of 4% (w/v) NaOH, very carefully washed with water until no more change in the 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.

For conductimetric measurements, the hydrochloride and perchlorate salts of chitosan were obtained in situ by dissolving the polymer with exact equivalent amount of the corresponding acid. Therefore they were diluted with water until the desired concentration was attained.

2.2.2. Purification of polygalacturonic acid

An aqueous $5\ \mathrm{g\ l}^{-1}$ solution of sodium polygalacturonate was stirred with ionic exchange resin Amberlite IR120 (H^+ form) during 24 h, after which the solution was filtered out and neutralised up to a pH ≈ 8 with 0.1 M NaOH. The solution was then successively filtered through sintered glass filter (pore diameter: 45 μm) and membranes (0.45 and 0.2 μm). The polysaccharide was precipitated by gradual addition of ethanol saturated with NaCl (approximately 0.3 M) until a final 70% (v/v) alcohol concentration was attained. The solid was separated by centrifugation and further washed with ethanol/water mixtures of increasing concentration: 80, 90 and 100% (v/v), until no change in the conductivity was detected. The purified sodium polygalacturonate was dried as proceeded with chitosan.

2.2.3. Conductimetric measurements

Conductimetric titrations were carried out in a glass cell at 25 ± 1°C under nitrogen atmosphere. A digital equipment HANNA INSTRUMENTS HI 8733 was employed. Previous to measurements the instrument was calibrated at the same temperature using a standard solution purchased from the same company. The pH-values of the polyelectrolyte solutions were those of the starting solutions.

3. Results and discussion

According to the theories of polyelectrolytes [15], a charged macromolecule is characterised by the so-called charge parameter, ξ, defined by

$$\xi = \frac{\mathrm{e}^2}{\varepsilon \mathrm{k} T b}.$$

In this expression e is the charge of the electron, ε the dielectric constant, b the average distance between charges along the polymer chain and kT the Boltzmann term.

As the charge parameter is proportional to the charge density, it depends on the chemical composition of the polymer. For weak polyelectrolytes, it varies with the degree of ionisation of the ionizable groups which depends on the pH and concentration of the solution.

For chitosan, the charge parameter is given by [13]:

$$\xi = 1.38(1 - \mathrm{DA})\alpha$$

where DA is the degree of *N*-acetylation and α the fraction of charged functional groups. The value of b was assumed to be 5.15 Å.

The corresponding relation for polygalacturonic acid is:

$$\xi = 1.61 \cdot (1 - \mathrm{DE}) \cdot \alpha$$

where DE is the degree of esterification. In this case, b was taken as 4.38 Å [16].

The specific conductivity, σ, of a polyelectrolyte solution is expressed by

$$\sigma = 1000 C f (\lambda_\mathrm{p} + \lambda_\mathrm{c})$$

In this formula λ_p and λ_c are the limiting ionic conductivities for the polyelectrolyte and the corresponding counterion, respectively, C is the concentration of the polyelectrolyte (expressed in equiv l^{-1}) and f is the transport coefficient. In the Manning theory, the latter is directly related to the charge parameter by the following relations [17]:

$$f = \begin{cases} 0.87/\xi & \text{if } \xi > 1, \\ 1 - \dfrac{0.55\xi^2}{(\pi + \xi)} & \text{if } \xi < 1. \end{cases}$$

3.1. Conductimetric analysis of chitosan hydrochloride

In this work, one proposes to investigate the complex formed between oppositely charged polyelectrolytes using conductivity measurements. We take into account the role of the presence of NaCl liberated when the complex is formed. Then, when a solution of chitosan hydrochloride is added to a NaCl aqueous solution with the same concentration as that of the polyanion to be used and expressed in

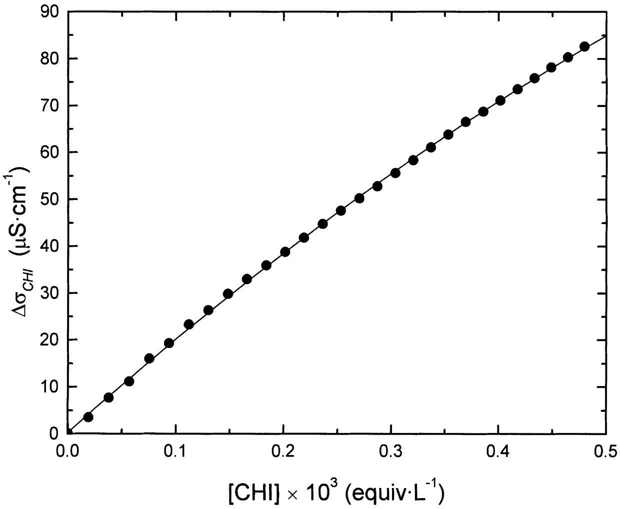

Fig. 1. Specific conductivity (expressed as $\Delta\sigma_{\mathrm{CHI}}$) as a function of chitosan concentration when the CHI solution (4.59×10^{-3} equiv l^{-1}) is added to NaCl solution (4.59×10^{-4} equiv l^{-1}). The solid curve corresponds to the quadratic function fitted to experimental data.

equiv l^{-1}, the total specific conductivity of the system will equal the conductivity of the chitosan solution plus that of the sodium chloride. If we take into account the following dissociation equilibrium:

$$\underset{(1-\alpha')}{\sim\!\sim NH_3^{+}\,{}^{-}Cl} + H_2O \rightleftharpoons \sim\!\sim NH_2 + \underset{\alpha'}{H_3O^{+}} + \underset{\alpha'}{Cl^{-}} \qquad (I)$$

where α' is the degree of hydrolysis; then, the conductivity of chitosan hydrochloride solution could be expressed as:

$$\Delta\sigma_{\mathrm{CHI}} = \sigma_{\mathrm{T}} - \sigma_{\mathrm{NaCl}} = C\alpha'(\lambda_{\mathrm{H}^+} + \lambda_{\mathrm{Cl}^-}) + C(1-\alpha')f_{\mathrm{CHI}}(\lambda_{\mathrm{P}^+} + \lambda_{\mathrm{Cl}^-}) \qquad (1)$$

where λ_{P^+}, λ_{Cl^-} and λ_{H^+} are the equivalent ionic conductivities of the polycation, the chloride ions and the hydrogen ions, respectively. Here σ_{T} is the total conductivity of the system as experimentally measured, while σ_{NaCl} is that of the NaCl solution, which could be estimated theoretically taking into account its concentration at each point due to dilution during titration.

Eq. (1) could be simplified as:

$$\frac{\Delta\sigma_{\mathrm{CHI}}}{C} = P_2 \qquad (1')$$

where

$$P_2 = \alpha'(\lambda_{\mathrm{H}^+} + \lambda_{\mathrm{Cl}^-}) + (1-\alpha')f_{\mathrm{CHI}}(\lambda_{\mathrm{P}^+} + \lambda_{\mathrm{Cl}^-}).$$

The result obtained when this experiment was carried out is illustrated in Fig. 1. There it could be appreciated a slight tendency of the slope to diminish as the polyelectrolyte concentration is increased due to the decrease of α' as a consequence of equilibrium I.

Hereinafter P_2 will denote the first derivative of σ_{CHI} with respect to C. As an alternative method it could be theoretically calculated if the values of the transport coefficient, f_{CHI}, and the limiting ionic conductivity of chitosan, λ_{P^+}, are known.

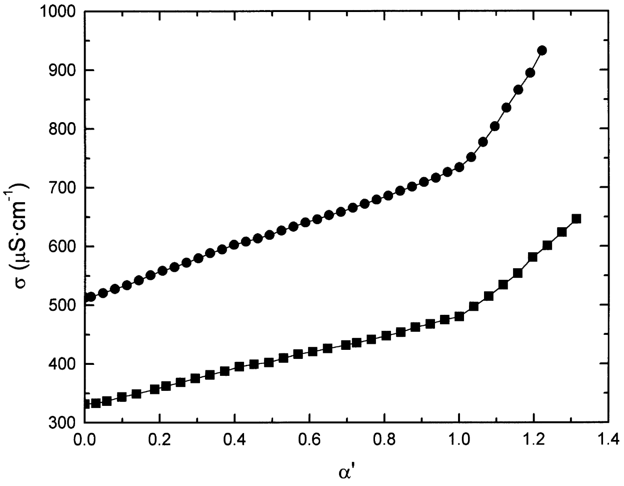

Fig. 2. Conductimetric curves corresponding to the addition of NaOH solution to chitosan hydrochloride (●, 5.81×10^{-3} equiv l^{-1}) and chitosan perchlorate (■, 4.07×10^{-3} equiv l^{-1}) solutions as a function of the degree of neutralisation, α'. The values of specific conductivity have been corrected in order to take into account the dilution during the gradual addition of the solution.

In order to evaluate these parameters, solutions of hydrochloride and perchlorate salts of chitosan were titrated with NaOH aqueous solution (Fig. 2). The values of the specific conductivities of the starting solutions could be expressed as:

$$\sigma_{1\mathrm{CHI}} = C_1 f_{\mathrm{CHI}}(\lambda_{\mathrm{P}^+} + \lambda_{\mathrm{Cl}^-}) \qquad (2)$$

$$\sigma_{2\mathrm{CHI}} = C_2 f_{\mathrm{CHI}}(\lambda_{\mathrm{P}^+} + \lambda_{\mathrm{ClO4}^-}) \qquad (3)$$

their difference being

$$\frac{\sigma_{1\mathrm{CHI}}}{C_1} - \frac{\sigma_{2\mathrm{CHI}}}{C_2} = f_{\mathrm{CHI}}(\lambda_{\mathrm{Cl}^-} - \lambda_{\mathrm{ClO4}^-})$$

then

$$f_{\mathrm{CHI}} = \frac{\sigma_{1\mathrm{CHI}}/C_1 - \sigma_{2\mathrm{CHI}}/C_2}{(\lambda_{\mathrm{Cl}^-} - \lambda_{\mathrm{ClO4}^-})}. \qquad (4)$$

From Eq. (4) it could be readily estimated the transport coefficient, *f*, of the polyelectrolyte and then, substituting its value in Eq. (2) or (3) λ_{P^+} could also be calculated.

The values obtained for chitosan were $f_{\mathrm{CHI}} = 0.82$ and $\lambda_{\mathrm{P}^+} = 31.4 \times 10^{-4}$ m^2 S mol^{-1}, which are similar to those found for poly(L-lysine) [12]. The theoretical value for the transport coefficient estimated according to Manning for 80% deacetylated chitosan is 0.79, which is very close to the experimental one.

3.2. Conductimetric analysis of sodium polygalacturonate

The same procedure followed for chitosan can be used for NaPGA. The weak polyelectrolyte character of NaPGA

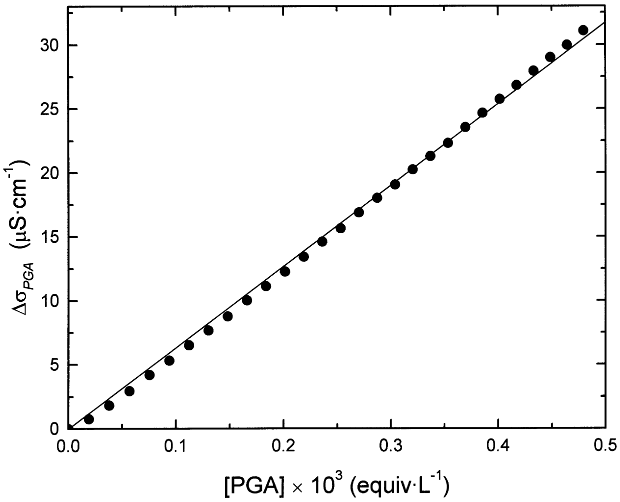

Fig. 3. Specific conductivity (expressed as $\Delta\sigma_{PGA}$) as a function of sodium polygalacturonate concentration when the NaPGA solution (4.59×10^{-3} equiv l^{-1}) is added to NaCl solution (4.59×10^{-4} equiv l^{-1}). The straight line corresponds to the linear regression fitted to experimental data.

could be represented as:

$$\sim\sim\underset{\alpha}{\text{COO}^-}\,{}^+\text{Na} + H_2O \rightleftharpoons \sim\sim\underset{1-\alpha}{\text{COOH}} + \underset{1-\alpha}{\text{OH}^-} + \underset{1-\alpha}{\text{Na}^+} \qquad \text{(II)}$$

In this case

$$\Delta\sigma_{PGA} = \sigma_T - \sigma_{NaCl} = C(1-\alpha)(\lambda_{Na^+} + \lambda_{OH^-}) + C\alpha f_{PGA}(\lambda_{P^-} + \lambda_{Na^+}). \qquad (5)$$

Here σ_T is the total conductivity of the system as experimentally determined, while σ_{NaCl} is that of the NaCl solution, which is calculated taking into account the concentration at each point during its progressive addition, and

$$\frac{\Delta\sigma_{PGA}}{C} = P_1 \qquad (5')$$

where

$$P_1 = (1-\alpha)(\lambda_{Na^+} + \lambda_{OH^-}) + \alpha f_{PGA}(\lambda_{P^-} + \lambda_{Na^+}).$$

In Fig. 3 are shown the conductimetric measurements when the NaPGA solution is added to a solution of NaCl. However, it should be noticed that in this case, there is almost a linear dependence of conductivity upon dilution.

The calculation of λ_{P^-} was carried out using a similar procedure to that illustrated for chitosan, but measuring the conductivity for lithium and potassium polygalacturonate at the equivalence point ($\alpha = 1$). For this purpose, the sodium polygalacturonate solution was passed through a cationic exchange resin under H^+ form (IR120), and the resulting PGA solution was then titrated with LiOH and KOH, respectively. Experimental curves are shown in Fig. 4.

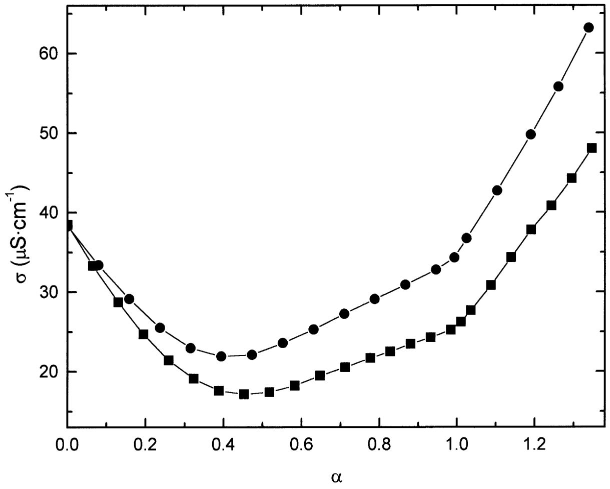

Fig. 4. Conductimetric curves corresponding to the addition of KOH (●) and LiOH (■) solutions (3×10^{-3} equiv l^{-1}) to the polygalacturonic acid solution (3.45×10^{-4} equiv l^{-1}) as a function of the degree of neutralisation, α. The values of specific conductivity have been corrected in order to take into account the dilution during the gradual addition of the solution.

Based on these measurements it was estimated that $f_{PGA} = 0.64$ and $\lambda_{P^-} = 56.8 \times 10^{-4}$ m^2 S mol^{-1}. These data are in good agreement with those found by Thibault et al. [16] for a sample of polygalacturonic acid with similar chemical composition. Moreover, the value found for the transport coefficient is almost the same as that calculated from theoretical considerations ($f_{PGA} = 0.62$), taking into account the total content of galacturonic acid (87.5%).

3.3. *Conductimetric analysis of the reaction between CHI and NaPGA*

Fig. 5 shows the behaviour of the conductivity of the reaction mixture as a function of the molar fraction of the reaction system, Z([CHI]/[PGA]), during the formation of the polyelectrolyte complex when chitosan solution is added to a solution of NaPGA. As the value of Z is increased it could be appreciated a slight and monotonic increment in the slope of the conductivity curve. After the equivalence point it is observed a change in the slope of the conductivity curve of the system, indicating that the formation of PEC has been completed and an excess of chitosan hydrochloride begins to appear in the reaction medium.

At the equivalence point it was estimated that Z([CHI]/[PGA]) $\cong 1.0$. Experiments carried out formerly in our laboratory on this system by viscometry, potentiometry, turbidimetry and gravimetry have been shown that this PEC exhibits a value of Z([CHI]/[PGA]) equal to 0.98 ± 0.04 at the equivalence point, no matter what order of mixing was chosen, which is in good agreement with this result [18].

Potentiometric measurements carried out simultaneously in our laboratory have shown that the pH of the reaction medium does not change appreciably during the addition of CHI to NaPGA until values of Z very near to unity. Indeed, it varies from 6.6 at $Z = 0$ to approximately 6.1 at Z slightly

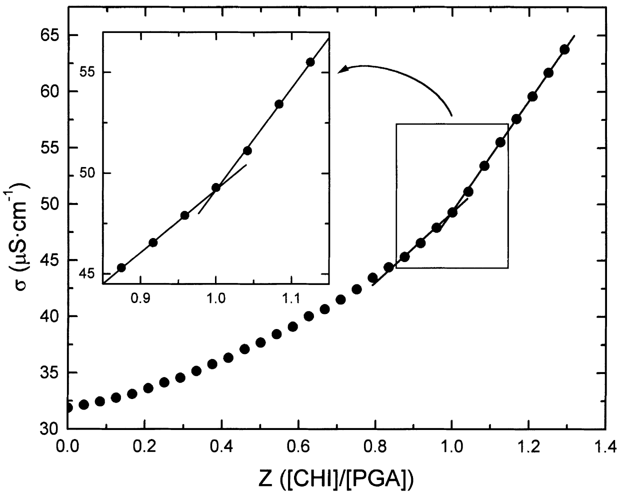

Fig. 5. Conductimetric curve corresponding to the addition of chitosan hydrochloride solution (4.59×10^{-3} equiv l^{1}) to that of sodium polygalacturonate (4.59×10^{-4} equiv l^{-1}) as a function of the molar fraction of the reaction system, *Z*([CHI]/[PGA]). Inside the figure the experimental points in the vicinity of the equivalence point are shown enhanced. The values of specific conductivity have been corrected in order to take into account the dilution during the gradual addition of the solution.

higher than 0.9 (Ref. [18]). Then it could be considered that during the formation of the PEC the polyacid is almost fully ionised.

The interpolyelectrolyte interaction when chitosan hydrochloride reacts with sodium polygalacturonate could be represented as:

$$\underset{(1\text{-}Z\theta)}{P^{-}Na^{+}} + \underset{Z(1\text{-}\theta)}{P^{+}Cl^{-}} \rightleftharpoons \underset{Z\theta}{P^{-}P^{+}} + \underset{Z\theta}{Na^{+}} + \underset{Z\theta}{Cl^{-}} \tag{III}$$

with θ being the degree of complexation for the formation of the complex $P^{+}P^{-}$ having a null charge. Taking into account additivity of the contributions of all ionic species, the conductivity of the reaction medium could be represented according to

$$\sigma(Z) = C(1 - Z\theta)P_1 + CZ(1 - \theta)P_2 + CZ\theta P_3. \tag{6}$$

In the above expression the equilibria I and II have been taken into account and

$$P_3 = \frac{\sigma_{\mathrm{NaCl}}}{C} = (\lambda_{\mathrm{Na}^+} + \lambda_{\mathrm{Cl}^-}) = \text{Constant}.$$

As it could be observed the parameter P_3 could be estimated theoretically and experimentally by a simple way. In this case, we opted for the theoretical value ($P_3 = 126 \times 10^{-4}$ m^2 S mol^{-1}), which is very close to the experimental one (121×10^{-4} m^2 S mol^{-1}).

If $\theta < 1$, the values of f_{CHI} and f_{PGA} will change as a function of θ, going to 1 when θ is large; this effect is due to the decrease of the effective charge density of the polyelectrolytes. In addition, f_{PGA} value will depend also on Z because the formation of the polyelectrolyte complex will significantly decrease the concentration of uncomplexed carboxylic groups. As a consequence, it should be necessary to admit that P_1 and P_2 depend on the degree of titration. Nevertheless, as a first approximation, one may assume that they remain constant.

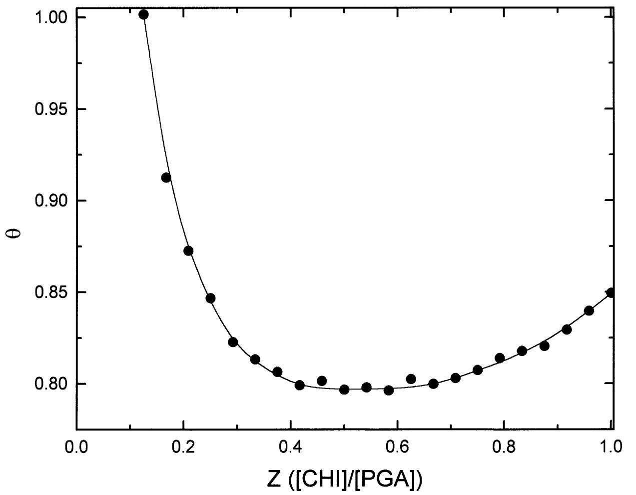

Fig. 6. Values of degree of complexation calculated by Eq. (11) as a function of the molar fraction of the reaction system, *Z*([CHI]/[PGA]).

On the one hand, this could be considered as the hypothetical situation in which by mixing both polyelectrolytes no complex is formed ($\theta = 0$). The theoretical approximation to this condition can be described by the sum of Eqs. (1′) and (5′):

$$\sigma_{\theta=0}(Z) = CP_1 + CZP_2 \tag{7}$$

which coincides with Eq. (6) if we let $\theta = 0$.

On the other hand, an additional limiting case has to be taken into account when $\theta = 1$. In this case, Eq. (6) could be written as:

$$\sigma_{\theta=1}(Z) = C(1 - Z)P_1 + CZP_3$$

and for $Z = 1$, it becomes:

$$\sigma_{\theta=1}(Z = 1) = C(\lambda_{\mathrm{Na}^+} + \lambda_{\mathrm{Cl}^-}) = CP_3.$$

In this case, the conductivity is only due to the NaCl formed by ion pairing. Then, subtracting expression (5′) from Eq. (6)

$$\frac{\sigma(Z) - \Delta\sigma_{\mathrm{PGA}}}{C} = Z\theta(\lambda_{\mathrm{Cl}^-} + \lambda_{\mathrm{Na}^+} - P_1 - P_2) + ZP_2$$

and dividing by Z

$$\frac{\sigma(Z) - \Delta\sigma_{\mathrm{PGA}}}{CZ} = \theta(P_3 - P_1 - P_2) + P_2. \tag{8}$$

If $\theta = 1$, when $Z < 1$

$$\frac{\sigma_{\theta=1}(Z) - \Delta\sigma_{\mathrm{P}^-}}{CZ} = P_3 - P_1. \tag{9}$$

Then the difference between expressions (7) and (6) could be calculated by

$$\frac{\sigma_{\theta=0}(Z) - \sigma(Z)}{ZC} = \theta(P_1 - P_3 + P_2). \tag{10}$$

It must be noted that formulae (8) and (10) give us the possibility to estimate the degree of complexation, θ, for soluble polyelectrolyte complexes if the values of P_1, P_2 and P_3 are known.

Nevertheless, it could be easily demonstrated that when the PEC resulting from the interaction of oppositely charged polymers is insoluble, the ionic species undergoing phase separation should not be taken into account in the analysis of the conductimetric behaviour. Then, expression (6) denoting the dependence of the specific conductivity of the reaction mixture is converted to:

$$\sigma(Z) = C(1 - Z)P_1 + CZ\theta P_3, \qquad \text{for } Z \leq 1.$$

Here we neglect the contribution of a fraction (CZ) of the polyacid present, as well as all the polycation added. This is because, we assumed that for $Z \leq 1$ all the polyelectrolytes that have been incorporated into the PEC do not contribute to the general conductivity of the reaction mixture. In this particular situation,

$$\theta = \frac{\sigma(Z) - C(1 - Z)P_1}{CZP_3} \tag{11}$$

which allows one to determine the degree of complexation for different compositions of the reaction mixture.

In order to evaluate P_1 the following procedure was accomplished: the dependence of the conductivity on the concentration for NaPGA alone was fitted to a linear function by a least square procedure (see the straight line in Fig. 3). P_1 is estimated as $63.5 \times 10^{-4}\ \mathrm{m}^2\ \mathrm{S}\ \mathrm{mol}^{-1}$.

In Fig. 6 the values of degree of complexation estimated by Eq. (11) 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 are almost completely complexed, with θ values falling very rapidly to very 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 and is equal to 0.85 for $Z = 1$.

The values for the degree of complexation of the interpolyelectrolyte reaction between chitosan and polygalacturonic acid determined here are quite high, which reflects that under selected experimental conditions this system behaves cooperatively.

References

[1] Kawashima Y, Handa T, Kasai A, Takenaka H, Lin SY, Ando A. Novel method for the preparation of controlled release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate. J Pharm Sci 1985;74:264–268.

[2] Dubin P, Bock J, Davis RM, Schulz DN, editors. Macromolecular complexes in chemistry and biology. New York: Springer, 1994 chap. 15, 17 and 18.

[3] Bell CL, Peppas NA. Biomedical membranes from hydrogels and interpolymer complexes. Adv Polym Sci 1995;122:125–175.

[4] Cascone MG, Sim B, Downes S. Blends of synthetic and natural polymers as drug delivery systems for growth hormone. Biomaterials 1995;16:569–574.

[5] Kikuchi Y, Fukuda H. Polyelectrolyte complex of sodium dextran sulphate with chitosan. Makromol Chem 1974;175:3593–3596.

[6] Chavasit V, Kienzle-Sterzer C, Torres J. Formation and characterization of an insoluble polyelectrolyte complex: chitosan–polyacrylic acid. Polym Bull 1988;19:223–230.

[7] Skorikova YeYe, Vilkhoreva GA, Kalyuzhnaya RI, Zezin AB, Gal'braikh LS, Kabanov VA. Polyelectrolyte complexes on chitosan basis. Vysokomol Soedin 1988;A30:44–49.

[8] Argüelles-Monal W, Gárciga M, Peniche-Covas C. Study of the stoichiometric polyelectrolyte complex between chitosan and carboxymethyl cellulose. Polym Bull 1990;23:307–313.

[9] Peniche-Covas C, Argüelles-Monal W, San Román J. Sorption and desorption of water vapour by membranes of the polyelectrolyte complex of chitosan and carboxymethyl cellulose. Polym Int 1995;38:45–52.

[10] Pérez-Gramatges A, Argüelles-Monal W, Peniche-Covas C. Thermodynamics of complex formation of poly(acrylic acid) with poly(*N*-vynil-2-pyrrolidone) and chitosan. Polym Bull 1996;37:127–134.

[11] Yao KD, Liu J, Cheng GX, Lu XD, Tu HL, Lopes Da Silva JA. Swelling behavior of pectin/chitosan complex films. J Appl Polym Sci 1996;60:279–283.

[12] Domard A, Rinaudo M. Polyelectrolyte complexes: interaction between poly(L-lysine) and polyanions with various charges densities and degrees of polymerization. Macromolecules 1981;14:620–625.

[13] Rinaudo M, Milas M, Le Dung P. Characterisation of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. Int J Biol Macromol 1993;15:281–285.

[14] Garnier C, Axelos MA, Thibault JF. Phase diagrams of pectin-calcium systems: influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of methylation. Carbohydr Res 1993;240:219–232.

[15] Lifson S, Katchalsky A. The electrostatic free energy of polyelectrolyte solutions. II-Fully stretched macromolecules. J Polym Sci 1954;13:43–55.

[16] Thibault JF, Rinaudo M. Interactions of mono- and divalent counterions with alkali- and enzyme-deesterified pectins in salt-free solutions. Biopolymers 1985;24:2131–2143.

[17] Manning GS. Limiting laws and counterion condensation in polyelectrolyte solutions. II. Self-diffusion of the small ions. J Chem Phys 1963;51:934–938.

[18] Cabrera G. Estudio del complejo polielectrolito entre la quitosana y el ácido poligalacturónico. MSc thesis, Universidad de La Habana, 1998, p. 37–9.