Conductimetric study of the interpolyelectrolyte reaction between chitosan and pectin

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Recibido: 26 de diciembre de 2011. Aceptado: 3 de mayo de 2012.

Palabras clave: quitosana, pectina, polisacárido, complejo polielectrolito, conductimetría.
Key words: pectin, polysaccharide, polyelectrolyte complex, conductimetry.

RESUMEN. Se realizó el estudio de la reacción interpolimérica entre dos polielectrolitos naturales: la quitosana, polisacárido catiónico y la pectina, polisacárido de naturaleza aniónica. La reacción intepolielectrolítica se siguió a través de la variación de la conductividad de una disolución de pectina ($2{,}03 \cdot 10^{-4}$ equiv $\cdot$ L^{-1}) resultante de la adición de una disolución de hidrocloruro de quitosana ($3{,}55 \cdot 10^{-3}$ equiv $\cdot$ L^{-1}) debido a la formación del complejo polielectrolito (CPE). La aparición de una ligera turbidez en la disolución durante la titración evidenció la formación del CPE insoluble. El cambio de pendiente de la curva de titración después del punto de equivalencia indica que se ha completado la reacción interpolielectrolítica. De esta forma, se pudo determinar la estequiometria del complejo, Z ([Quitosana]/[Pectina]), que resultó Z = 0,89. La extensión de la reacción o grado de acomplejamiento, θ, fue evaluado en función de la relación [Quitosana]/[Pectina] y se mantuvo cerca de la unidad en todo el intervalo estudiado, evidenciando el elevado grado de cooperatividad de la reacción. El mayor grado de acomplejamiento se obtuvo con las primeras adiciones del hidrocloruro de quitosana a la disolución de pectinato de sodio ($\theta = 0{,}98$ a Z = 0,24). A partir de entonces, θ decreció hasta un valor mínimo ($\theta = 0{,}94$) a $Z \approx 0{,}6$, para incrementar nuevamente hasta casi la unidad ($\theta = 0{,}99$) a Z = 0,83.

ABSTRACT. The study of the interpolymer reaction between two natural polelectrolytes, chitosan, a cationic polysaccharide, and pectin a polysaccharide of anionic nature is undertaken. The interpolyelectrolyte reaction was followed by monitoring the variation of the conductivity of a pectin solution ($2.03 \cdot 10^{-4}$ equiv $\cdot$ L^{-1}) resulting from the addition of a chitosan hydrochloride solution ($3.55 \cdot 10^{-3}$ equiv $\cdot$ L^{-1}) due to the formation of a polyelectrolyte complex (PEC). The appearance of a slight turbidity in the solution during titration evidenced the formation of the insoluble PEC. The slope change in the titration curve after the equivalence point indicates that the interpolyelectrolye reaction has been completed. Therefore, it was possible to determine the stoichiometry of the complex, Z ([Chitosan]/[Pectin]), which turned out to be Z = 0.89. The extent of the reaction or degree of complexation, θ, was evaluated as a function of the [Chitosan]/[Pectin] ratio. It remained close to unity throughout the whole [Chitosan]/[Pectin] interval studied, evidencing that the reaction is highly cooperative. The highest degree of complexation was obtained with the first additions of chitosan hydrochloride to the sodium pectinate solution. From then on θ decreases reaching a minimum value ($\theta = 0.94$) at $Z \approx 0.6$ and then increases again to almost unity (= 0.99) at Z = 0.83.

INTRODUCTION

Polyelectrolyte complexes (PECs) result from the interaction between oppositely charged polyions.[1] They are environmentally responsive materials that find diverse applications in the biomedical field e.g., in drug delivery devices,[2] cell encapsulation,[3] in the fabrication of skin substitutes and scaffolds for tissue engineering.[4-6] Many of these applications use PECs composed of natural occurring polyelectrolytes, such as chitosan, alginate, pectin, carboxymethylcellulose, hyaluronic acid, chondroitin sulphate and pectin.[7-10]

Chitosan (CHI) is a copolymer of (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan and 2-amino-2-deoxy-β-D-glucan that can be obtained by extensive alkaline deacetylation of chitin, the second most abundant polysaccharide in nature.[11] Chitosan is biocompatible, biodegradable and non-toxic. Therefore, it has been considered as a very valuable material in biomedical, pharmaceutical and agricultural applications, among others.[2,3,12,13] Chitosan is insoluble in alkaline and neutral pH. At low pH, amine groups of CHI become protonated, with a resultant positively charged soluble polysaccharide ($R\text{-}NH_3^+$), with an extended configuration because of the ionic repulsion between cationic groups. Due to its unique cationic character, chitosan interacts with polyanions generating polyelectrolyte complexes.

Pectin, and pectic substances are complex copolymers made of $\alpha(1\rightarrow4)$ polygalacturonic acid units, which can be partially esterified with methoxyl groups.[14] The linear chain in pectin macromolecule is usually interrupted by rhamnopyranosyl residues in adjacent or alternate positions. Pectin is widely used in the food, cosmetic and drug industries, and in medical treatments as a stabilizer and gelling ingredient.[15]

Chitosan readily reacts with pectin yielding polyelectrolyte complexes.[16] Chitosan/pectin PECs have been proposed for tablet coating, in the form of aqueous film-coating dispersion or as a blended powder, or as matrix in drug release devices.[17-20]

The extent of complex reaction and the stoichiometry of the resulting PEC depend on the properties of the starting polyelectrolytes (position and properties of the ionic groups, charge density and rigidity of the chains).[21] Moreover, both parameters, the extent of complex reaction and the stoichiometry of the resulting PEC are influenced by the solution pH, temperature, ionic strength, solvent and the chemical environment of the reactive sites.[22]

PEC formation always involves the release to the reaction medium of low molecular weight electrolytes. For that reason, conductimetric titration is a useful method for evaluating the extent of the reaction and determining the complex stoichiometry. This technique has been successfully applied in the study of the reaction of chitosan with sodium alginate[23] and polygalacturonic acid.[24] The aim of the present work is to carry out the inter-polyelectrolyte reaction between chitosan and pectin in order to determine the extent of the reaction and the stoichiometry of the resulting PEC when a chitosan hydrochloride solution is added to a pectin solution.

MATERIALS AND METHODS

Chitosan from lobster cephalotorax (Panulirus argus) was obtained in author's laboratory. The viscosity average molecular weight, estimated at 25 ± 0.01 ºC in 0.3 mol/L acetic acid/0.2 mol/L sodium acetate was $\overline{M}_v = 1.27 \cdot 10^5$ g · mol^{-1} and the degree of N-acetylation DA = 0.18 was determined by ^{1}H NMR.

Pectin obtained from orange peels was supplied by Sigma Chemical Co. Average molecular weight, determined by viscometric measurements in 0.1 mol/L sodium chloride, was $\overline{M}_v = 3.74 \cdot 10^4$ g · mol^{-1}. Galacturonic acid content (GA = 40.7 %) and degree of esterification (DE = 59.2 %) were determined by conductimetric measurements as described elsewhere.[25] Prior to use, both polymers were thoroughly purified. All the experiments were carried out using twice distilled water ($\sigma \approx 3$ µS·cm^{-1}). The rest of chemicals were analytical grade.

Methods

Purification of chitosan

Chitosan solution (5 g · L^{-1} dissolved in 1 % (v/v) acetic acid) 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). Precipitation was accomplished by drop-wise addition of 1 mol/L NaOH until pH ≈ 9. The precipitate was carefully washed with water until no change in conductivity was detected, and then washed successively with ethanol at concentrations 70, 80, 90 and 100 % (v/v), respectively. The purified polymer was finally dried in a vacuum at room temperature.

Purification of pectin

An aqueous solution of pectin (2.5 g · L^{-1}) was successively filtered through glass wool, sintered glass filters (pore diameters: 45 and 5 µm) and membranes (0.8 and 0.45 µm). The filtered solution was passed throughout an ionic exchange resin, Amberlite IR120 (H^+ form). The solution was further neutralized up to a pH near 8 with 1 mol/L NaOH in order to convert the polyacid into its salt form. NaCl was added to reach a final 0.1 mol/L 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 washed with ethanol-water mixtures of increasing concentrations 80, 90 and 100 % (v/v). The purified polymer was vacuum dried at room temperature.

Conductimetric titrations

Conductimetric titrations were carried out in a glass cell at 25 ± 0.1 °C. For that purpose a digital conductimeter Hanna Instruments HI 8733 was employed. The equipment was previously calibrated with a standard solution provided by the same company (σ = 12.88 µS · cm^{-1} at 25 °C).

RESULTS AND DISCUSSION

The specific conductivity (σ) of a polyelectrolyte solution is expressed by

$$\sigma = \Lambda C 1000 = f(\lambda_c + \lambda_p) \qquad (1)$$

Where: Λ is the equivalent conductivity of the polyelectrolyte,

C is the concentration (expressed in equiv · L^{-1}),

λ_p and λ_c are the limiting ionic conductivities of the polyelectrolyte and the corresponding polyion, respectively, and

f is the transport parameter, a quantity related to the charge parameter ξ, defined by:

$$\xi = \frac{e^2}{\varepsilon k T b} \qquad (2)$$

Where: e is the electron charge,

k is the Boltzmann constant,

T is the temperature,

ε is the dielectric constant of the solvent and

b is the distance between charges along the polymer chain, which has been assumed to be 0.515 nm for chitosan[26] and 0.435 nm for pectin.[27]

The parameter b is dependent on the degree of ionization of the polyelectrolyte —which depends on the pH and concentration of the solution— and on their degree of substitution, which in chitosan corresponds to the degree of acetylation (DA) and in pectin is the degree of esterification (DE). It has been shown that the charge parameter is given by:

$\xi = 1.38\ (1 - DA)\ \alpha$

for chitosan[26], and

$\xi = 1.61\ (1 - DE)\ \alpha$

for pectin[25].

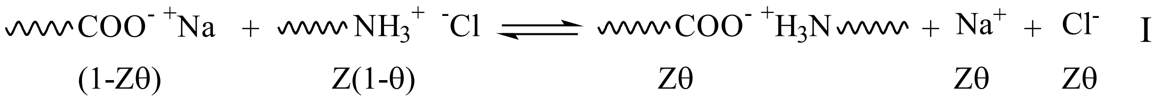
In the two expressions above, α stands for the fraction of charged functional groups in the polyelectrolyte.

The transport parameter values can be calculated from the theoretical relationships predicted from Manning's model:[28]

$$f = 0.87\ \xi \qquad f\ \ \xi > 1 \qquad (3) \qquad f = 1 - \frac{0.5\ \ \xi^2}{(\pi + \xi)}\ \ f\ \ \xi < 1 \qquad (4)$$

Conductimetric analysis of the interpolyelectrolyte reaction between chitosan and pectin

The interpolyelectrolyte reaction was followed by monitoring the conductivity of a pectin solution ($2.03 \cdot 10^{-4}$ equiv $\cdot$ L^{-1}) resulting from the addition of a chitosan hydrochloride solution ($3.55 \cdot 10^{-3}$ equiv $\cdot$ L^{-1}) due to PEC formation. The development of a slight turbidity in the solution evidenced the formation of the insoluble PEC. The reaction can be represented as follows:



$$\underset{(1-Z\theta)}{\sim\sim COO^{-}\ {}^{+}Na} + \underset{Z(1-\theta)}{\sim\sim NH_3^{+}\ {}^{-}Cl} \rightleftharpoons \underset{Z\theta}{\sim\sim COO^{-}\ {}^{+}H_3N \sim\sim} + \underset{Z\theta}{Na^{+}} + \underset{Z\theta}{Cl^{-}} \qquad I$$

Where:

θ is the degree of complexation –i.e. the fraction of functional groups of the polyelectrolyte reacted- and

Z is the molar ratio of polyelectrolytes in the reaction system (Z = [CHI]/[PECTIN]).

The evolution of the conductivity of the pectin solution as Z increases as a result of the addition of the chitosan hydrochloride solution was followed (Fig.1). The monotonic increase in the conductivity of the solution observed in the first part of the titration curve is caused by the release to the reaction media of sodium and chloride ions resulting form the interpolyelectrolyte reaction I. The slope change after the equivalence point indicates that the interpolyelectrolye reaction has been completed and that the further increase in conductivity is due to excess chitosan hydrochloride in solution.

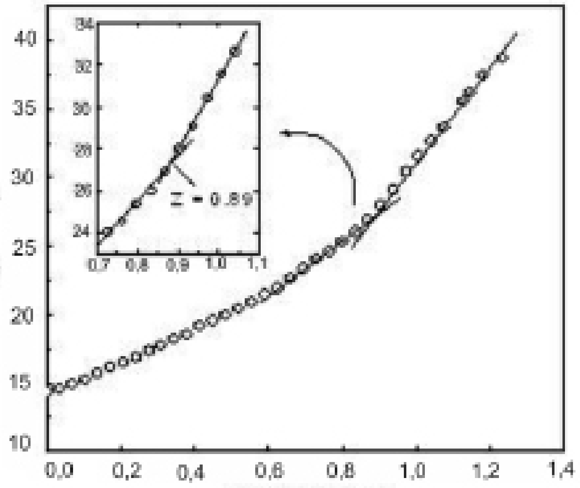


Fig.1. *Conductimetric titration curve of sodium pectinate (2,03 · 10^{-4} mol · L^{-1}) with chitosan hydrochloride (3,99 · 10^{-3} mol · L^{-1}).*

The estimated value of the complex composition at the equivalence point was

Z([CHI]/[PECTIN]) = 0.89 ± 0.04.

Therefore, it can be concluded that the chitosan/pectin PEC is a non-stoichiometric complex. This behavior is common to PECs formed between weak polylelectrolytes for which the stoichiometry of the complex is highly pH dependent, due to the variation of their dissociation degree with pH. Actually, 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 and

α_{PA} and α_{PB} are the respective dissociation degrees.[29]

Considering the additivity of the contribution of all ionic species to the conductivity of the solution, it can be expressed as:

$$\sigma(Z) = C(1 - Z\theta)P_1 + CZ\;(1-\theta)P_2 + CZ\;\theta P_3 \qquad (5)$$

Where: the parameters $P_1 = \frac{\Delta\sigma_{PECTIN}}{C}$, and $P_2 = \frac{\Delta\sigma_{CHI}}{C}$ represent the contribution to the specific conductivity of the solution of the unreacted units of sodium pectinate and chitosan hydrochloride, respectively. Hereinafter P_1 and P_2 will denote the first derivative of σ_{PECTIN} and σ_{CHI} with respect to C, in that order. P_3 is the contribution of the sodium chloride released to the reaction medium, which is given by:

$$P_3 = \frac{\sigma_{NaCl}}{C} = \left(\lambda_{Na^+} + \lambda_{Cl^-}\right) = \text{constant}$$

where:

λ_{Cl^-} and λ_{Na^+} are the equivalent ionic conductivities of chloride and sodium ions, respectively.

P_3 can be calculated either by experimental or theoretical methods. In the present work, the authors employed the theoretical value ($126.4 \cdot 10^{-4}$ m²·S · mol⁻¹).

The expressions for P_1 and P_2 must take into account the dissociation equilibria of both weak polyelectrolytes. Thus, for sodium pectinate

$$\sim\!\sim COO^{-}\,{}^{+}Na \;+\; H_2O \rightleftharpoons \sim\!\sim COOH \;+\; OH^{-} \;+\; Na^{+} \qquad II$$

$$\alpha \qquad\qquad\qquad 1-\alpha \qquad 1-\alpha \qquad 1-\alpha$$

and P_1 can be expressed as:

$$P_1 = (1-\alpha)\left(\lambda_{Na^+} + \lambda_{OH^-}\right) + \alpha f_{pect}\left(\lambda_{P^-} + \lambda_{Na^+}\right) \qquad (6)$$

where:

λ_{P^-} and λ_{OH^-} are polyanion and hydroxyl equivalent ionic conductivities, respectively and f_{pect} is the transport coefficient for the polyelectrolyte.

In order to evaluate P_1, the contribution to the conductivity of the solution of the NaCl liberated during complex formation was taken into account. The evolution of the conductivity obtained by adding sodium pectinate solution ($1.59 \cdot 10^{-3}$ equiv · L⁻¹) to a solution of NaCl ($4.00 \cdot 10^{-4}$ equiv · L⁻¹) was followed (Fig. 2). In every point of the curve (Fig. 2), the specific conductivity of the system will equal the conductivity of the pectinate solution plus that of the sodium chloride. Therefore, the conductivity of the sodium pectinate solution, σ_t, can be expressed as

$$\Delta\sigma_{PECTIN} = \sigma_t - \sigma_{NaCl} = C(1-\alpha)\left(\lambda_{Na^+} + \lambda_{OH^-}\right) + C\alpha f_{pect}\left(\lambda_{P^-} + \lambda_{Na^+}\right) \qquad (7)$$

P_1 can be evaluated from the slope of the straight line obtained by plotting the conductivity of NaCl solution with the sodium pectinate as a function of the sodium pectinate concentration. This was obtained by adding a sodium pectinate solution ($1.59 \cdot 10^{-3}$ equiv · L⁻¹) to the NaCl solution ($4.00 \cdot 10^{-4}$ equiv · L⁻¹). It can be appreciated that the variation of $\Delta\sigma_{PECTIN}$ with sodium pectinate concentration can be satisfactorily represented by a straight line (Fig. 2), and from its slope the P_1 value was estimated as $P_1 = 64.0 \cdot 10^{-4}$ m²·S · mol⁻¹.

Similarly, for chitosan hydrochloride:

$$\sim\!\sim NH_3^{+}\,{}^{-}Cl \;+\; H_2O \rightleftharpoons \sim\!\sim NH_2 \;+\; H_3O^{+} \;+\; Cl^{-} \qquad III$$

$$1-\alpha' \qquad\qquad\qquad\qquad \alpha' \qquad \alpha'$$

where:

α' is the degree of hydrolysis. Therefore, the contribution of chitosan hydrochloride to the conductivity of the solution can be expressed as

$$P_2 = \alpha'\left(\lambda_{H^+} + \lambda_{Cl^-}\right) + (1-\alpha')f_{CHI}\left(\lambda_{P^+} + \lambda_{Cl^-}\right) \qquad (7)$$

where:

λ_{P^+} is the equivalent ionic conductivity of the polycation, and

f_{CHI} is the transport coefficient of the polyelectrolyte.

However, in the CHI/pectin system the analysis can be simplified, because when an insoluble complex is formed the ions undergoing phase separation do not contribute to the total conductivity of the solution, and the equation

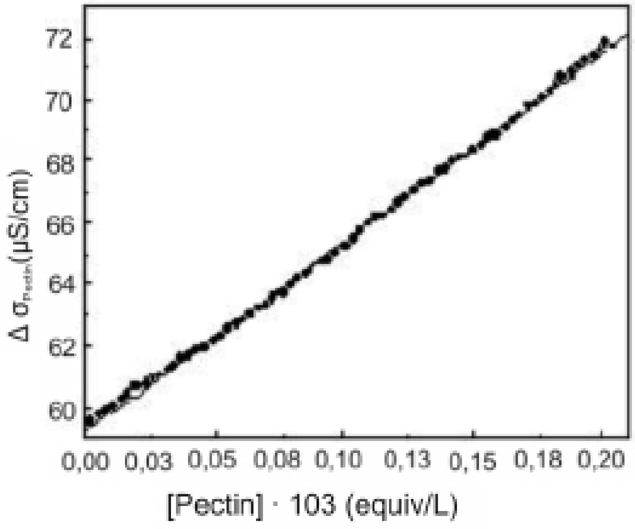


Fig. 2. Specific conductivity (Δ_{PECTIN}) as function of pectin concentration when a sodium pectinate solution ($1.59 \cdot 10^{-3}$ equiv · L^{-1}) is added to a NaCl solution ($4.00 \cdot 10^{-4}$ equiv · L^{-1}). The straight line corresponds to the linear least squeares fit of experimental data.

(5) reduces to:

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

In this expression the authors have assumed that the polyelectrolytes taking part in the polyelectrolyte complex formed do not contribute to the conductivity of the solution. Then

$$\theta = \frac{\sigma(Z) - C(1-Z)P_1}{CZP_3} \qquad (8)$$

Taking into account that the CHI/pectin PEC is non-stoichiometric (Z = 0.89), equation (8) transforms into equation (9):

$$\theta = \frac{\sigma(Z) - C(1 - Z/0.89)P_1}{CZP_3} \qquad (9)$$

Equation (9) allows estimating the degree of complexation as a function of Z (Fig. 3). It can be appreciated that the highest degree of complexation is obtained with the first additions of chitosan hydrochloride to the sodium pectinate solution (θ = 0.98 at Z = 0.24). From then on, θ decreases reaching a minimum value (θ = 0.94) at Z ≈ 0.6

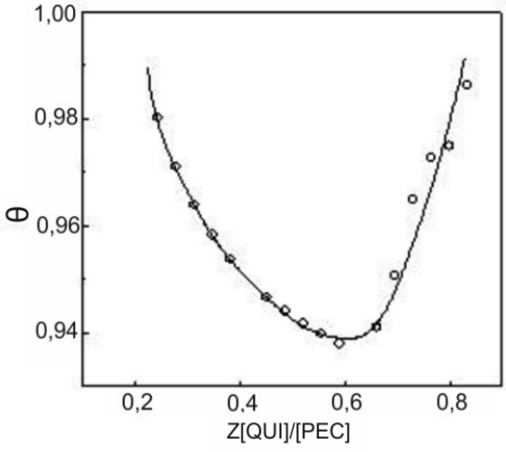


Fig. 3. Values of degree of complexation calculated by Eq. (9) as a function of the molar fraction of the reaction system, Z([CHI]/[PGA]).

and then increases again to almost unity ($\theta = 0.99$) at Z = 0.83. It is worth noting however, that for all the range of compositions the degree of complexation is very high. This contrasts with the results obtained from the PEC formed between chitosan and polygalacturonic acid. In the latter, even though the values of θ were also near unity for $Z \approx 0.2$, they decreased to a minimum $\theta = 0.80$ in the range for $Z \approx 0.4$ - 0.6, but experienced only a slight increment in the degree of complexation with increasing Z, reaching only 0.85 at stoichiometry (Z = 1).[23]

A possible explanation for the lower degree of complexation obtained for polygalacturonic acid when $Z \geq 0.6$ as compared with that found for pectin in the present work relies on the difficulty of the polygalacturonic chain to efficiently accommodate all its carboxylate groups (GA = 87.5 %) in the spatial position required to react with the $–NH_3^+$ groups of chitosan hydrochloride. Pectin, having a lower galacturonic acid content (GA = 40.7 %) should find it easier to reach a higher extent of conversion.

CONCLUSIONS

By means of conductimetric titrations it was possible to determine that the interpolyelectrolyte reaction between pectin (DE = 59.2 %) and chitosan (DA = 18.1 %) yields a non stoichiometric polyelectrolyte complex (Z [Chitosan]/[Pectin] = 0.89). It is pointed out, however, that changes in the pH of solutions will result in varying Z. The degree of complexation, (θ) was always near unity, which indicates that the reaction is highly cooperative. The lower galacturonic acid content of pectin (GA = 40.7 %) allows reaching higher degrees of conversion of the interpolyelectrolyte reaction at $Z \geq 0.6$ than the homologous polygalacturonic acid (GA = 87.5 %).

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