Supramolecular Systems from Natural Polymers and Maleic Polyelectrolytes

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Summary: The formation of polyelectrolyte complexes by interaction between chitosan and maleic acid copolymers as strong/weak dibasic polyanions was investigated. The interaction between the sodium salt of maleic acid copolymers with styrene or vinyl acetate and the chitosan hydrochloride in aqueous solution was followed by potentiometric, conductometric and turbidimetric titration. The effect of the added low molecular salt on the complex formation was also investigated. The precipitated complexes were analyzed by FTIR spectroscopy and TG analysis. Preliminary layer-by-layer deposition experiments were performed to obtain thin films.

Keywords: chitosan; layer-by-layer deposition; maleic acid copolymers; polyelectrolyte complexes; polyelectrolytes

Introduction

Polyelectrolyte complexes (PEC) result from the interaction of macromolecules carrying opposite charged groups. They have been proposed for several purposes, from which we mention the design of drug delivery systems, anticoagulant coatings, and membranes or skin substitutes. The preparation of PEC from natural polymers, such as polysaccharides, has the additional advantage of being non-toxic, biocompatible, and bioabsorbable.

Chitosan is the only cationic polysaccharide, obtained by deacetylation of chitin, which is the major constituent of the shells of crustacean and insects. It is renewable, highly biocompatible, very low toxic and biodegradable.[1,2] By derivatization or complexation of chitosan a variety of new interesting biomaterials can be obtained. The PECs of chitosan with natural/natural modified[3–12] or synthetic[13–15] polyanionic partners have been studied. Because of the antimicrobial, haemostatic properties of chitosan,[2] the films obtained from chitosan could be susceptible of the same properties or could be used as drug delivery systems. PEC films from maleic acid-vinyl(polyalkyleneoxy)methyl ether copolymer and chitosan were prepared by casting/solvent evaporation method and the properties of drug release were studied.[16–18]

In this paper we studied the PEC formation in aqueous solutinbetween chitosan and maleic acid copolymers (maleic polyelectrolytes – MP) as synthetic polyanions with a reproductible alternative structure, with two adjacent carboxylic groups on the repeating unit.[20,21] The interaction was studied by potentiometry, conductometry and turbidimetry, the combination of which had proved to be suitable for the investigation of PEC formation in the transition range to phase separation.[19] It was followed the influence of the chemical structure of maleic copolymer, of the mixing order, and of the added salt. The complex in solid form was studied by FTIR and thermogravimetric analysis. Preliminary layer-by-layer experiments were performed to obtain thin films from MP and chitosan hydrochloride.

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Experimental Part

Polymers

Chitosan was provided by Yaizu Suisankagaku Ind., Japan, with the characteristics given in Table 1. The degree of acetylation was determined by dissolving the chitosan in a known excess of HCl and performing potentiometric titration with 0.1 N NaOH. The average molar mass was determined by viscometric measurements with an Ubbelohde capillary viscometer ($\phi = 53$ mm) at 25 °C using 0.3 M acetic acid/0.2 M sodium acetate as solvent.[22] The chitosan was dissolved in water by adding a stoichiometric amount of HCl, in order to prepare the hydrochloride salt (ChCl) (Scheme 1). The ChCl solution was purified by diafiltration and freeze-drying to obtain the solid form which was used for recording the FTIR spectrum and TG analysis. As it could not be solved again in water, we used the solution purified by diafiltration, and its concentration was determined by potentiometric, conductometric and turbidimetric titration with 0.1 N NaOH.

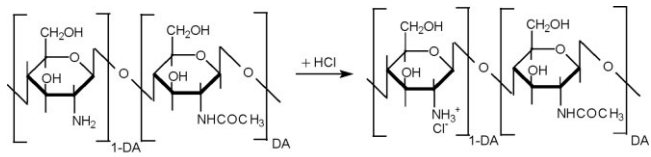
Copolymers of maleic anhydride with styrene and vinyl acetate were obtained by free-radical copolymerization.[20,23] The average molecular mass of the copolymers, given in Table 1, was determined by viscometric measurements in acetone at 30 °C[24,25] and the copolymers composition was determined by conductometric titration in acetone:water (1:1 vol) mixture. MP were prepared by the hydrolysis of the maleic anhydride copolymers and neutralization with diluted NaOH. The solutions were purified by diafiltration and freeze-drying, when the copolymers sodium maleate-styrene (AS) and sodium maleate-vinylacetate (AV) with the structure presented in Scheme 2 were obtained.

Table 1.
The physico-chemical characteristic of the polymer partners.

Polymer	Acetylation degree (%)	Composition, maleic anhydride: comonomer (moles)	Molecular weight
Chitosan	18	–	80 000
AS	–	1:1	104 000
AV	–	1:1	91 000

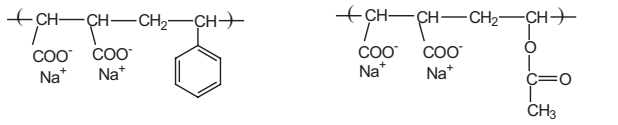
Methods

Potentiometric titrations were made with an all-purpose titrator 716DMS Titrino, Metrohm, equipped with a dosing unit and a 6.0214.010 combined glass electrode. Conductometric titration was made using



Chitosan — Chitosan hydrochloride (ChCl)

Scheme 1.
Chemical structure of chitosan and hydrochloride form.



Sodium maleate - styrene copolymer (AS) — Sodium maleate - vinyl acetate copolymer (AV)

Scheme 2.
Chemical structure of maleic polyelectrolytes.

a Radiometer CDM210 conductivity meter and a CDC 641T cell. Turbidity measurements were made with a Brinkmann PC 900 turbidimeter equipped with a 20-23-634-5 type fiber optic cell. The thermooxidative decomposition under dynamic conditions of heating has been performed with a Paulik-Paulik-Erdey Derivatograph MOM-Budapest on 50 mg samples, at 12 °C/min. The FTIR spectra have been recorded with a spectrophotometer FTIR Vertex 70 Bruker, in KBr pellets. The UV-vis absorption spectra were recorded on a SPECORD 200 UV-vis spectrophotometer.

Layer by Layer Deposition

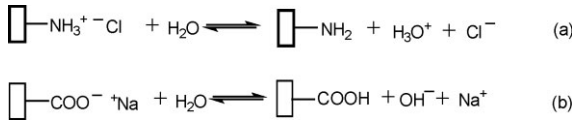
The substrate used was a quartz plate that was cleaned with freshly prepared piranha solution for 100 minutes, then washed with twice distilled water for several times. These treatments generated negative charge on the surface. For the multilayer preparation, the substrate was immersed in ChCl solution (2 mg/mL) for 15 min and then rinsed by repeatedly dipping in twice distillated water. It was then immersed in AS solution (2 mg/mL), washed by repeatedly dipping in twice distillated water and the process was repeated from the immersion in ChCl.

Results and Discussion

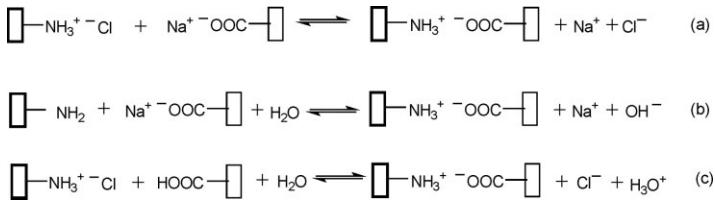
Chitosan hydrochloride, as weak cationic polyelectrolyte, and the sodium salt of maleic copolymers, as weak anionic polyelectrolytes, are not fully dissociated in solution according to the equilibria presented in Scheme 3. The electrostatic interaction between the oppositely charged polyelectrolytes can be developed according to Scheme 4.

PEC formation was followed by potentiometric and conductometric titration and the precipitation of PEC was followed by turbidimetric titration. The influence of the comonomer when ChCl was added to MP solution is illustrated in Figure 1. For both copolymers, the complex formation is visible by precipitation on the turbidimetric titration curves (Figure 1a). Similarly, on the conductometric titration curves (Figure 1b) a slope modification can be observed: firstly the conductivity increases due to the mobility increase of Na^+ and Cl^- ions, then the conductivity increases only due to the excess of added ChCl.

The potentiometric curves are different function of the comonomer. The PEC formation in the case of AS copolymer seems to be up to [ChCl]:[MP]. ~0.5 (the end point). After this value there is the



Scheme 3.
The dissociation equilibrium of chitosan hydrochloride (a) and MP (b).



Scheme 4.
Possible interactions between dissociated and undissociated ChCl and MP.

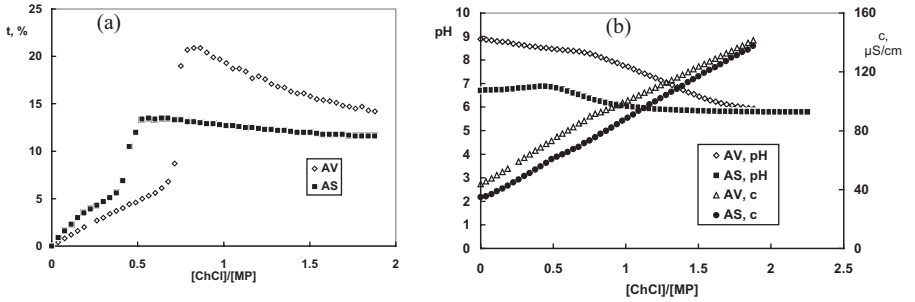


Figure 1.
Turbidimetric (a), potentiometric and conductometric titration curves (b) when 10^{-2} N solution of ChCl was added to 10^{-3} N solutions of MP.

excess part of the curve, manifested by slower increase of the conductivity, slow decrease of pH and constant values of turbidity. From all these data we can admit that before the end point the interactions 4a and 4b take place, then the addition of ChCl in excess produces the observed behavior. In the case of AV copolymer the potentiometric behavior is different, so we can ascribe the formation of complex according to Scheme 4a up to [ChCl]:[MP] ~ 0.8, accompanied by the reaction 4c. The three methods: turbidimetry, conductometry and potentiometry, are in good accordance, but the end points are not located at [ChCl]:[MP] = 1:1.

The influence of the added salt when ChCl was added to MP solution is presented in Figure 2. As it is well known, the added salt decreases the pH of the copolymer solution increasing the dissociation and favoring the PEC formation.

The influence of the MP structure when it was added to ChCl solution is presented in Figure 3. From the turbidimetric titration curves (Figure 3a) it is observed that the tendency of PEC formed with AV to solve again when an excess of MP is added is greater than that of the AS PEC, in concordance with Figure 1a.

The pH increase up to [MP]:[ChCl] = 1.5 and 1.8, respectively (Figure 3b) is due to the reaction from Scheme 4b. The turbidimetric and potentiometic titration are in good agreement, but the end points are also shift from [MP]:[ChCl] = 1 ratio towards higher values. The influence of the low molecular salt when MP was added to the ChCl solution is presented in Figure 4. In the first step we can observe the same behavior, the pH being shift to higher values due to the salt effect. In the second step the order of curves is reversed. With 0.1 M NaCl only a monotonous variation of

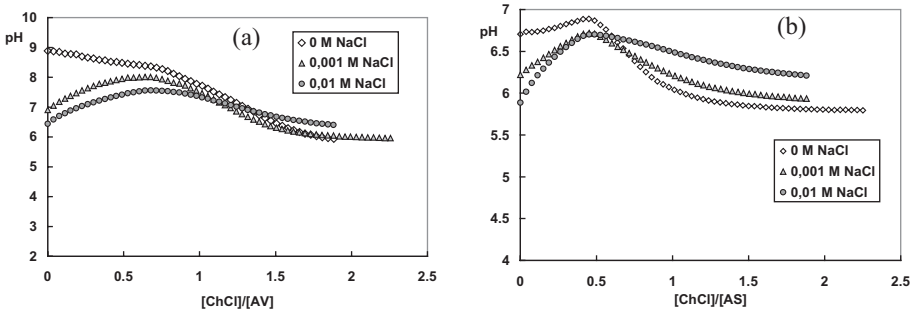


Figure 2.
Potentiometric titration curves when 10^{-2} N solution of ChCl was added to 10^{-3} N solutions of AV (a) and to 10^{-3} N solutions of AS (b) in the presence of different salt concentrations.

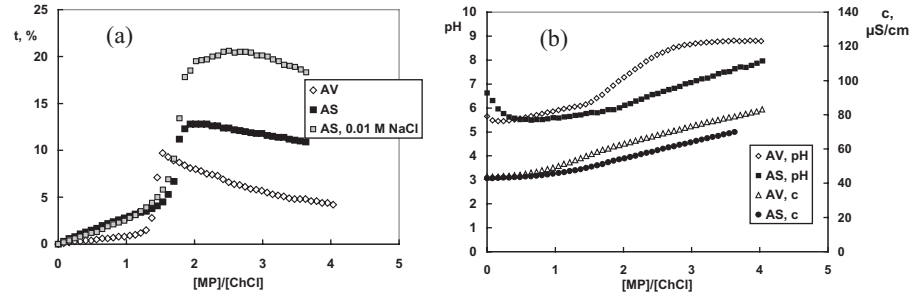


Figure 3.
Turbidimetric (a), potentiometric and conductometric titration curves (b) when 10^{-2} N solution of MP were added to 0.5×10^{-3} N solutions of ChCl.

pH is observed, possibly due to the screening of coulombian interactions produced by salt.

The influence of the mixing order on the turbidimetric curves is summarized in Figure 5. The mixing order do not influence the value of [ChCl]/[MP] at the end points. For the same copolymer, the different maximum turbidity is due to the variation of the concentration.

The PEC prepared from ChCl and AS at the end point was recovered by centrifugation, dried at 40 °C under low pressure and characterized by FTIR spectra and thermogravimetric analysis. The spectra of AS, ChCl, physical mixture and PEC were compared. The absorption band at 1719 cm^{-1} corresponding to the –COOH groups is present in the PEC spectrum but not into one of the mixture, and that was observed in ChCl – carboxymethylcellulose[3,5,10] or carboxymethyldextran[4] complexes. The band around 2050 cm^{-1} corresponding to NH_3^+, is diminished in the PEC spectrum. These both suggest either that the complexation would favor the formation of the undissociated –COOH and $–NH_2$ groups or the reversibility of the PEC formation according to the Scheme 5:[10]

Thermal analysis of PEC, physical mixture of ChCl with AS and of the partners is presented in Figure 6. Considering the temperature at which the rate of thermal degradation is highest (T_{max}), the thermal stability decreases according to the series:

$$\begin{aligned} &AS(T_{max} = 293\,^{\circ}C) > PEC(T_{max} = 253\,^{\circ}C) \\ &> \text{mixture}(T_{max} = 247\,^{\circ}C) \\ &> ChCl(T_{max} = 234\,^{\circ}C) \end{aligned}$$

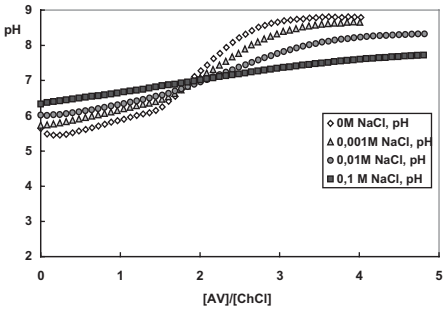


Figure 4.
Potentiometric titration curves when AV 10^{-2} N solution was added to the ChCl solution $0{,}5 \times 10^{-3}$N, in the presence of different concentrations of added salt.

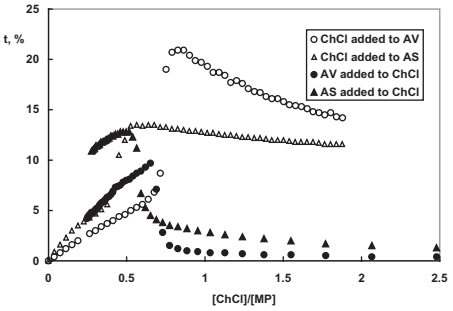
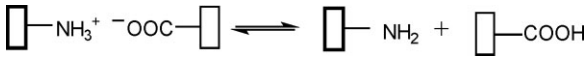


Figure 5.
The influence of the mixing order of the turbidimetric titration curves.



Scheme 5.
The equilibrium between the PEC and free partners.

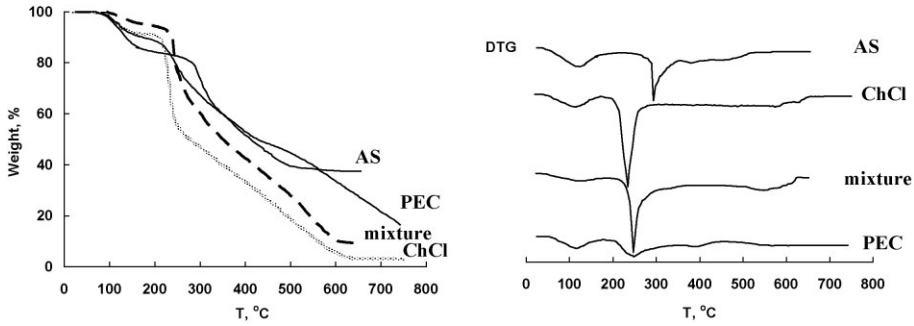


Figure 6.
TG and DTG curves of ChCl, AS, mixture and PEC.

The increasing of the thermal stability of PEC is due to the strong interaction between the two partners in the polyelectrolyte complex. This is confirmed by the fact that the weight loss of the complex is smaller than the mixture.

Based on these results, preliminary layer-by-layer experiments were performed to obtain thin films using AS as polyanion and ChCl as polycation (we chose the AS copolymer because it has an absorption band in UV range). The growth of the layers was monitored by means of UV spectroscopy as shown in Figure 7.

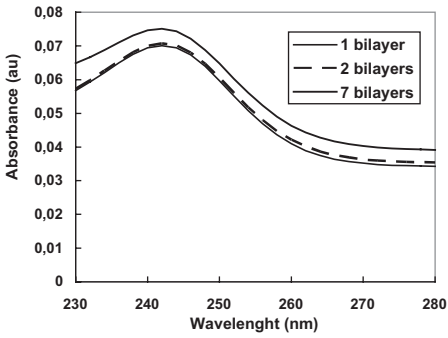


Figure 7.
UV absorption spectra on quartz substrate of multilayer ChCl/AS film.

Conclusion

The interaction of chitosan with maleic polyelectrolytes in aqueous solution was followed by potentiometric, conductometric and turbidimetric titration by varying the mixing order and the maleic polyelectryte structure (comonomer). The three methods are generally in agreement. The end points are shift from 1:1 ratio between the partners towards higher amounts of maleic copolymer, suggesting that only the first carboxylic groups were involved. The effect of added salt was clearly observed and could be ascribed to the enhanced complex formation the screening of electrostatic interactions or to a salting out effect (additional work is in progress to clarify these aspects).

The precipitated complex from chitosan hydrochloride and sodium maleate-styrene copolymer was obtained in solid form and analyzed by FTIR and thermal analysis. FTIR spectra evidenced that complexation would favor the formation of the undissociated COOH and NH_2 groups or the reversibility of the PEC formation. The strong interaction between the partners determined the increasing of the PEC thermal stability.

Preliminary layer-by-layer deposition experiments to obtain thin films from sodium maleate-styrene copolymer and chitosan hydrochloride were performed for the first time and the layer deposition was demonstrated by UV spectroscopy.

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