

Polyelectrolyte complex between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid)

O. Stoilova, N. Koseva, N. Manolova*, I. Rashkov

Institute of Polymers, Bulgarian Academy of Sciences, BG-1113 Sofia, Bulgaria

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Summary

A new polyelectrolyte complex between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid) (PAMPS) was prepared by mixing aqueous solutions of its components or by free radical polymerization on chitosan template. The complex formation was sensitive to the ionic strength of the medium. The complex was stable in acidic and neutral medium and dissociated at $\text{pH} > 8$. Its composition did not depend on pH values of the medium and the way of preparation. The mixing dilute aqueous solutions of chitosan and PAMPS resulted in the formation of nanoparticles with mean particle diameter 250 nm and monomodal distribution.

Introduction

The electrostatic attraction between anionic and cationic polyelectrolytes results in the formation of polyelectrolyte complexes (PECs) (1). PECs are generally obtained either by mixing solutions of polycations and polyanions or by polymerizing monomers having suitable functional groups onto template polymer. The formation of PECs is affected by the characteristics of its components (charge density and rigidity of the polymer chain, position of the ionic sites) and the chemical environment (pH, ionic strength, temperature, concentration) (2). The investigations of polyelectrolyte interactions may assist the understanding of biological phenomena due to their similarities to some biological assemblies (3). PECs have potential applications as membranes, medical implants, microcapsules and preparations for controlled drug delivery (4-6).

Chitosan is increasingly attracting interest as a polycationic partner in the formation of PECs. Chitosan is a biocompatible and biodegradable natural polyaminosaccharide with low toxicity (7), soluble in dilute acids (8). Chitosan based PEC with natural or synthetic polymers as sodium alginate (11), κ -carrageenan (12), polyacrylic acid (13, 14), polystyrene sulfonate (13), poly(4-N-methacrylamidobenzoic acid) (15) have been reported.

* Corresponding author

Poly(2-acryloylamido-2-methylpropanesulfonic acid) (PAMPS) bearing sulfonic acid groups belongs to the class of strong polyelectrolytes which typically have high degrees of ionization (16). The PECs formation of PAMPS with synthetic polymers (poly(2-vinylpyridine) and poly(4-vinylpyridine) is known.

In the present work the preparation and characterization of a new PEC composed of chitosan and PAMPS are discussed. The complex chitosan-PAMPS has been obtained by mixing chitosan and PAMPS aqueous solutions or by radical polymerization of 2-acryloylamido-2-methylpropanesulfonic acid onto chitosan template.

Experimental

Materials

High molecular chitosan (MW $6 \cdot 10^5$ with 0.80 deacetylation degree) and 2-acryloylamido-2-methylpropanesulfonic acid (AMPS) were purchased from Fluka. All salts used for the redox-initiator system ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, Na_2SO_3 , $(\text{NH}_4)_2\text{S}_2\text{O}_8$) and those for the preparation of buffer solutions were of analytical grade. The following buffer solutions were used: pH 1.5 (HCl/KCl); pH 3.8, 4.5 and 5.6 ($\text{CH}_3\text{COOH}/\text{NaOH}$); pH 7.0 and 8.0 ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$); pH 9.0 and 10.0 ($\text{NaHCO}_3/\text{Na}_2\text{CO}_3$).

PAMPS was prepared by free radical polymerization in 20% aqueous solution of the monomer at 25°C for 15 h applying redox-initiator system - $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ - 0.05 g/l, Na_2SO_3 - 2.2 g/l and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ - 2.2 g/l. The unreacted monomer was removed by dialysis against deionized water for 3 days. The product was dried under reduced pressure. The conversion was 95%. PAMPS molar mass, $5 \cdot 10^5$ g.mol⁻¹, was calculated from the intrinsic viscosity values, measured in 5M aqueous NaCl at 25°C with an Ubbelohde viscometer using the known Mark-Houwink constants values $\alpha = 0.80$ and $K = 2.11 \times 10^{-5}$ (16).

Measurements

The quantity of the PEC obtained by mixing 0.2% solutions of chitosan and PAMPS in buffer with pH 4.5 was evaluated gravimetrically. The individual polymer solutions were mixed at the following volumetric proportions (ml chitosan : ml PAMPS): 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 and thermostated in a shaker bath at 25°C for 48 h. White precipitate formed immediately upon mixing. The precipitates were isolated by centrifugation for 40 min at 5000 rpm and washed three times with deionized water. The PECs were dried to constant weight and analyzed by elemental analysis. The viscosity of the supernatant solutions was measured with an Ubbelohde viscometer at 25±0.1°C. The obtained results (yield of complex and viscosity of the supernatant solutions) are presented as function of the mole fraction of the aminoglycoside units in the feed: $M = A/(A+B)$, where A are the moles of aminoglycoside units; B - moles of AMPS-units.

The effect of pH and the ionic strength of the medium on the complex formation was examined turbidimetrically. Solutions of chitosan and PAMPS of concentrations 0.02% in buffer solutions with different pH: 1.5; 3.8; 4.5; 5.6 and ionic strength $I = 0.1$ were mixed varying the unit mole ratio of the partners in the feed. Similar measurements were performed for polymer mixtures with different ionic strength ($I = 0.1; 0.2; 0.5; 0.75; 1.0$) at pH 4.5. In order to determine the ionic strength at which the complex formation is

suppressed, measurements were also performed at $I = 2.0$ and 2.5 . The ionic strength was adjusted by addition of NaCl. The turbidity of the mixtures (absorbance at 420 nm) was registered on a UV-VIS spectrophotometer Specord 71, Germany. Turbidity data given in the figures are average values from three measurements.

The PEC stability was studied by stirring samples of a pre-formed complex chitosan-PAMPS for 100 h at 25°C in buffer solutions with pH 7.0, 8.0, 9.0 and 10.0. Then the mixtures were centrifuged and the remaining solids were washed repeatedly with deionized water and dried to constant weight.

The IR spectra of PAMPS, chitosan and their complex were registered on spectrophotometer FT-IR Bruker Vector 22 using KBr pellet technique.

The diameter of the particles obtained under certain conditions was determined by quasi-elastic light scattering measurements (QELS) using a He-Ne laser ($\lambda = 633$ nm) as a light source.

Template polymerization of AMPS in the presence of chitosan

The template polymerization of AMPS in the presence of chitosan was carried out in an aqueous solution at 25°C. Chitosan was dissolved in monomer solution in ratio [aminoglucoside units]:[AMPS] = 1:1 and total concentration of the solution 0.2%. The components of the redox-initiator system, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, Na_2SO_3 and $(\text{NH}_4)_2\text{S}_2\text{O}_8$, were added at stirring. The polymerization was completed for 24 h. The complex was obtained as a white precipitate, isolated by centrifugation, washed repeatedly with deionized water and dried to constant weight. Yield - 99%.

Results and discussion

Preparation of the complex chitosan-PAMPS by template polymerization

It was found that chitosan dissolved in an aqueous solution of AMPS in concentrations allowing protonation of more than 50% of NH_2 -groups in chitosan macromolecules. Thus the template polymerization was performed directly in the monomer solution. A redox initiator-system was used in order to avoid heating. The turbidity of the solution and the formation of a precipitate confirm that polymerization of AMPS on the chitosan template proceeds. From the precipitate composition determined by elemental analysis it was calculated that the ratio [aminoglucoside units] : [AMPS-units] in the complex was close to unity.

The IR spectrum of chitosan showed the following characteristic bands : 3500-3300 cm^{-1} (NH- and OH- stretching vibrations), 1651 cm^{-1} (Amide I), 1577 cm^{-1} (Amide II) and 1377 cm^{-1} (CH_2 -bending). The bands due to its saccharide structure were observed at 1153, 1030 and 1077 cm^{-1} . The bands were in accordance with literature (15, 17). The main bands in the PAMPS spectrum were as follows: 3420-3090 cm^{-1} - NH- and OH-stretching vibrations, 1647 cm^{-1} - Amide I, 1552 cm^{-1} - Amide II and 1393 cm^{-1} - CH_2 -bending. The bands due to the presence of sulfonic acid groups were in the range 1224-1153 cm^{-1} (18).

The IR spectrum of the complex distinguished from those of the polymers alone by the shift of the bands in the region due to the sulfonic acid groups towards higher frequencies (1200 cm^{-1} and 1144 cm^{-1}). A change in their intensities was also observed -

for the complex the stronger band was at 1144 cm^{-1} . In addition, a broad shoulder appears at *ca.* 2500 cm^{-1} which might be assigned to the NH_3^+ band (19). These findings are indicative of the existence of a strong interaction between chitosan and PAMPS most probably through ionic interaction between the amino groups of the former and the sulfonic acid groups of the latter. The characteristic bands in the ranges $3440\text{--}3083\text{ cm}^{-1}$ and $1550\text{--}1651\text{ cm}^{-1}$ could not be used to confirm the complex formation since they were present in the spectra of both polymers.

Complex formation by mixing chitosan and PAMPS solutions

The viscosity of chitosan and PAMPS solutions increased on increasing concentration. A precipitate was formed on mixing chitosan solution with PAMPS solution. The viscosity of the supernatant depended on the mole ratio of the partners in the feed (Figure 1). The supernatant specific viscosity reached a minimum at equimolar ratio [aminoglycoside

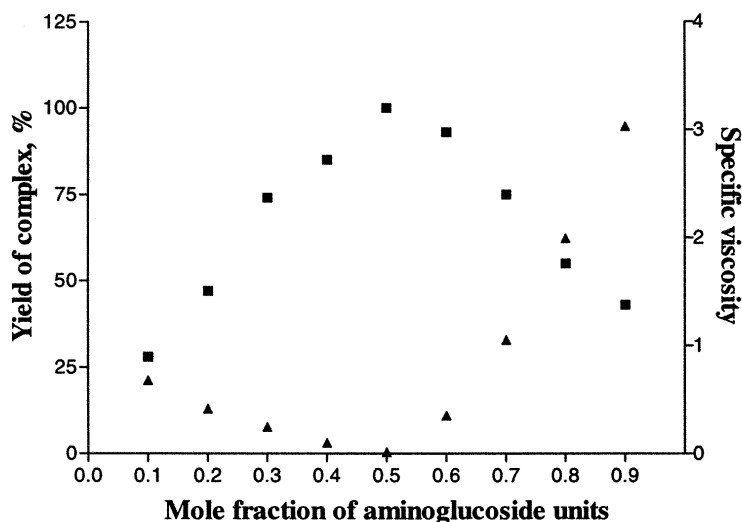


Figure 1. Dependence of the supernatant specific viscosity (▲) and the complex yield (■) on the mole fraction of aminoglycoside units in the feed (0.2% w/w); pH 4.5, at $25\text{ }^{\circ}\text{C}$

units] : [AMPS-units]. At that point the supernatant viscosity was almost equal to that of the medium. The concentrations of the uncomplexed chitosan or PAMPS were actually zero due to the quantitative formation of an insoluble PEC.

The complex formation was confirmed by gravimetric measurements. The dependence of the complex yield related to the initial polymer weight on the mole fraction of the aminoglycoside units (M) in the feed mixtures is shown in Figure 1. The amount of the isolated PEC was maximum at equimolar ratio [aminoglycoside units]:[AMPS-units]. These data demonstrated that at pH 4.5 and 25°C the interaction between chitosan and PAMPS resulted in PEC formation with quantitative yield and composition close to the stoichiometric one. The IR spectrum of the complex was very similar to the spectrum of the complex prepared by template polymerization.

The complex prepared by mixing dilute aqueous solutions of chitosan and PAMPS (concentrations 0.02%) was in the form of nanoparticles with mean particle diameter 250 nm and monomodal distribution. The particles did not form aggregates and did not change their size on storage in aqueous solution at 23°C during two weeks.

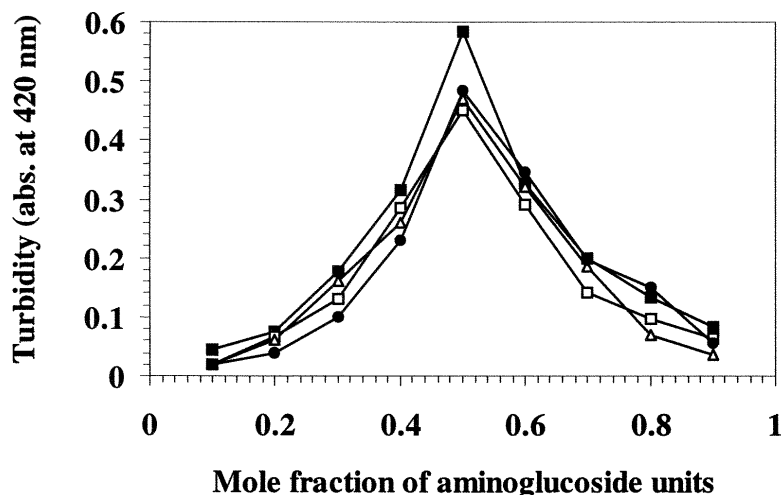


Figure 2. Dependence of the mixture turbidity on the mole fraction of aminoglycoside units at different pH values of the feed; (0.02% w/w); $I = 0.1$; 25°C
 pH 1.5 (■)
 pH 3.8 (□)
 pH 4.5 (△)
 pH 5.6 (●)

The effect of pH and ionic strength of the medium on the complex formation

The turbidity of the mixtures of chitosan and PAMPS solutions prepared under certain conditions (without PEC precipitation) was measured. The effect of pH on the complex formation was studied in the range 1.5 - 5.6 since at $\text{pH} > 5.6$ the probability for (co)precipitation of chitosan increases because of its insolubility in neutral and alkaline

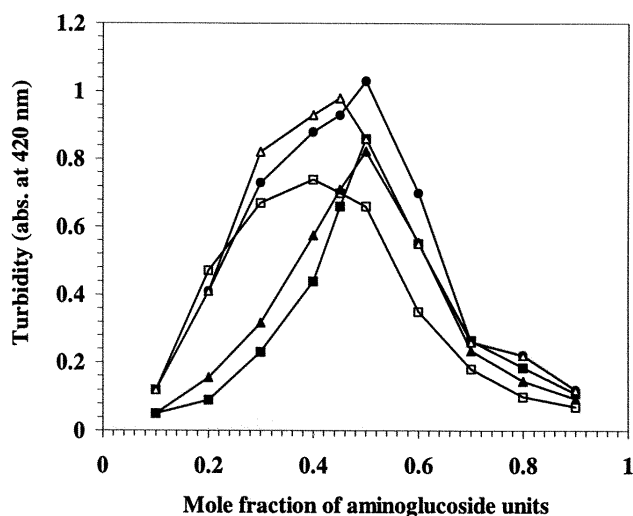


Figure 3. Dependence of the mixture turbidity on the mole fraction of aminoglycoside units at different ionic strength values of the feed (0.03% w/w); $\text{pH} 4.5$; 25°C
 $I = 0.1$ (■)
 $I = 0.2$ (▲)
 $I = 0.5$ (●)
 $I = 0.75$ (△)
 $I = 1.0$ (□)

solutions. At $I = 0.1$ the turbidity was maximum at equimolar ratio [aminoglycoside units] : [AMPS-units] (Figure 2). Using the pK_a value of chitosan ($\text{pK}_a = 6.5$ (20)) the degree of ionisation of chitosan NH_2 -groups was calculated. It is 1.0 at $\text{pH} 1.5$ and 0.9 at $\text{pH} 5.6$. The second partner, PAMPS, is a strong polyacid and is completely ionized at even low pH values. These facts explain the absence of measurable influence of pH of the medium on the complex formation. The enhanced complexation was more pronounced only in the

case of pH 1.5. A possible reason for this difference is the protonation of the N-atom from the NHCOCH_3 -group occurring at $\text{pH} < 3.5$ (21).

The complex stability in neutral and alkaline medium was examined. The weight of the samples did not change after being stirred for 100 h at pH 7.0 and 8.0 while in the case of pH 9.0 and 10.0 the samples lost 50% of their mass. The solubility in acetate buffer solution of the undissolved residues isolated at pH 9 and 10 implies that they were built up of chitosan. Therefore chitosan-PAMPS complex is stable up to pH 8; above this pH value dissociation of the complex proceeds.

The effect of the ionic strength of the medium on the complex formation is illustrated in Figure 3. The increase of the ionic strength caused a shift from the stoichio-

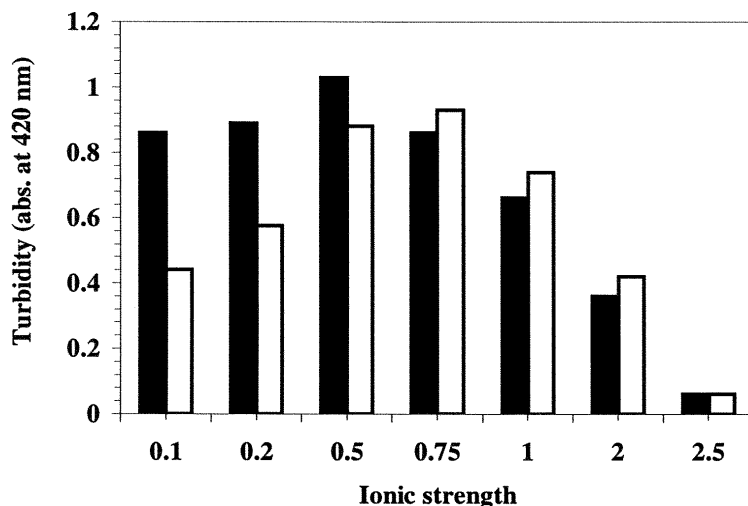


Figure 4. Dependence of the mixture turbidity on the ionic strength of the medium at feed molar ratio chitosan : PAMPS = 1:1 (□) and 2:3 (■); pH 4.5; 25 °C.

metric ratio [aminoglycoside units]:[AMPS-units] and the complex composition was enriched in polyanionic component. It might be considered that the possibility for formation of loops by PAMPS chains upon complexation with chitosan increases. This is due to the flexibility of PAMPS macromolecules which is better expressed at higher ionic strength of the medium. At different polyanion/polycation ratio in the feed the amount of complex formed was maximum at different ionic strength (Figure 4). For example, at ratio [aminoglycoside units]:[AMPS-units] = 1:1 the maximum amount of complex was obtained at $I = 0.5$, while at ratio [aminoglycoside units]:[AMPS-units] = 2:3 it was at $I = 0.75$. With further increase of the ionic strength the amount of the complex decreased and at $I = 2.5$ it was very small. The exact ionic strength value at which the complex formation was entirely suppressed could not be determined because the chitosan solution became turbid at $I = 3.0$.

In conclusion, a new polyelectrolyte complex between chitosan and PAMPS has been obtained and characterized. It is stable under acidic and neutral conditions and dissociates at $\text{pH} > 8$. The yield and complex characteristics are slightly affected by the pH change in the studied pH interval from 1.5 to 5.6, while they are quite sensitive to changes in the ionic strength of the medium. Investigations concerning the possible application of the complex for preparation of controlled drug delivery systems are in progress.

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