*e-Polymers* **2003**, no. 033.

http://www.e-polymers.org
ISSN 1618-7229

# Novel polyelectrolyte complex between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid-*co*-acrylic acid)

*Dilyana Paneva*, *Olya Stoilova*, *Nevena Manolova**, *Iliya Rashkov*

Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, Sofia 1113, Acad. G. Bonchev Str. bl. 103 A, Bulgaria;
Fax +359 2 8707 523; manolova@polymer.bas.bg

(Received: June 5, 2003; published: July 1, 2003)

*Abstract:* A novel polyelectrolyte complex between chitosan and copolymers of 2-acryloylamido-2-methylpropanesulfonic acid (AMPS) and acrylic acid (AA) has been prepared. The formation of the complex has been studied viscometrically, gravimetrically and turbidimetrically in the pH range from 1.2 to 5.8. The stoichiometry and the yield of the complex depend on the copolymer composition and on the pH value of the medium. In the case of copolymers with low content of AMPS units the complexes are enriched in copolymer when formed in the pH range from 1.2 to 4.8. In this pH region mainly AMPS units take part in complex formation. A stoichiometric complex forms only at higher pH values due to the increased number of complexable carboxylate ions of AA units. The stoichiometry of the complexes prepared from copolymers with higher content of AMPS units is close to equimolar and is less sensitive to pH. The obtained complexes are stable up to pH 8. It has been shown that chitosan once included in the complexes remains degradable under the action of a crude enzyme complex produced by the soil fungus *Trichoderma viride*. The rate of the enzymatic hydrolysis decreases in the order chitosan/PAA > chitosan/P(AMPS-*co*-AA) > chitosan/PAMPS. Tests on the proliferation of *T. viride* embedded in chitosan beads have shown that coating the beads with chitosan/P(AMPS-*co*-AA) complex does not hamper the development of the microorganisms.

## Introduction

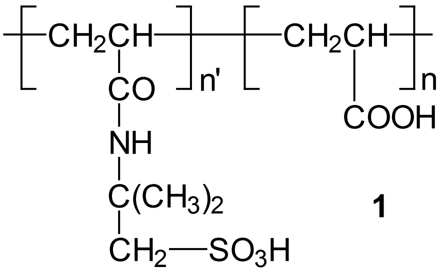
The preparation of polyelectrolyte complexes (PECs) of chitosan by mixing polymer solutions is a simple and convenient way for creating new polymer materials that might be used in agriculture and medicine. Thus, chitosan properties are combined with those of suitable synthetic polymers opening new prospects for its utilisation and valorisation as a polymer from renewable resources. Complexes based on chitosan are well known in the field of biomaterials, owing to their possible application for the immobilisation of enzymes, microencapsulation of cells, and for the design of drug release devices [1-3]. Complex formation between chitosan and polyacrylic acid (PAA) has been investigated by several groups [4-10]. The composition of the complexes chitosan/PAA depends on the pH value of the medium and a water-insoluble complex is formed in a narrow pH range – from 3 to 6 [10]. Previously, we have studied the PEC between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid) (PAMPS) [11]. Since PAMPS is a strong polyacid, the composition of the complex does not depend on the pH value of the medium and a water-insoluble complex with equimolar stoichiometry forms in a broad pH range – from 1.5 to 5.6. It was of interest to investigate the formation of polyelectrolyte complexes between chitosan and copolymers of AMPS and AA. Such copolymers have been reported to form interpolymer complexes stabilised through hydrogen bonds with the non-ionogenic polymers polyoxyethylene and poly(*N*-vinylpyrrolidone) [12].

The present study reports on the formation of polyelectrolyte complexes by mixing aqueous solutions of chitosan with copolymers of AMPS and AA. The dependence of the stoichiometry of the complexes on the pH value of the medium and on the composition of the copolymers is studied and the possibility of their application as carriers of microorganisms is tested.

## Results and discussion

### *Preparation and characterisation of P(AMPS-co-AA)*

Copolymers **1**, P($AMPS_{n'}$-*co*-$AA_n$), with n' = 0.1, 0.2, 0.5, 0.8 and n = 0.9, 0.8, 0.5, 0.2, where n' and n are the mole fractions of AMPS and AA units, respectively, were prepared by radical copolymerisation.




**1**

The molecular-weight characteristics of the copolymers obtained by size-exclusion chromatography and copolymer compositions estimated from elemental analysis and by comparing the signals in the $^{13}$C NMR spectra attributed to carbonyl carbon atoms of AMPS and AA units are presented in Tab. 1.

Tab. 1. Mole fraction of AMPS units in the copolymers and molecular-weight characteristics of the copolymers

| Sample | AMPS in copolym. in mol-% | | Molecular weight of copolymer [a], ×10$^{-3}$ | | | Polydispersity |
|---|---|---|---|---|---|---|
| | Elem. analysis | $^{13}$C NMR | $\overline{M}_n$ | $\overline{M}_v$ | $\overline{M}_w$ | $\overline{M}_w/\overline{M}_n$ |
| P($AMPS_{0.1}$-*co*-$AA_{0.9}$) | 10 | - | 60 | 120 | 131 | 2.2 |
| P($AMPS_{0.2}$-*co*-$AA_{0.8}$) | 20 | 20 | 58 | 127 | 144 | 2.5 |
| P($AMPS_{0.5}$-*co*-$AA_{0.5}$) [b] | 40 | 50 | > 315 | > 1600 | - | - |
| P($AMPS_{0.8}$-*co*-$AA_{0.2}$) | 70 | 80 | 53 | 141 | 152 | 2.9 |

[a] Size-exclusion chromatography data.

[b] The sample was partially excluded by the column set.

The IR spectra of copolymers showed the following characteristic bands: 3500 - 3300 $cm^{-1}$ (NH- and OH-stretching vibrations), 1720 $cm^{-1}$ (C=O of AA unit stretching vibration), 1647 $cm^{-1}$ (amide I), 1552 $cm^{-1}$ (amide II) and 1393 $cm^{-1}$ ($CH_2$ bending). The bands due to the presence of $-SO_3H$ groups of AMPS units were in the range 1224 - 1153 $cm^{-1}$. The absorption intensity of the band due to the carboxylic groups of AA units decreased with increasing content of AMPS units in the copolymers.

### *Complex formation between chitosan and P(AMPS-co-AA)*

In contrast to AMPS, which is a strong acid and is completely ionised in aqueous solutions, the ionisation of AA depends on the pH of the medium. Therefore, it might be expected that the composition and the yield of the complexes between chitosan and P(AMPS-*co*-AA) at different pH values would be determined by the content of ionised carboxylic groups of AA.

It was found that on mixing solutions of chitosan and P(AMPS-*co*-AA) white precipitate formed due to PEC formation. Complex formation was studied viscometrically, turbidimetrically and gravimetrically as a function of pH of the medium and of the composition of the copolymers. The viscosity of the supernatant decreased, and the decrease depended on the mole ratio of the partners (Fig. 1). In the case of P($AMPS_{0.1}$-*co*-$AA_{0.9}$) the viscosity of the supernatant as a function of the mole fraction of aminoglucoside units was minimum at a mole ratio [aminoglucoside units] : [copolymer units] = 0.1 : 0.9. At the minimum point the viscosity was close to that of the solvent since the concentrations of free chitosan and free copolymer in the supernatant were close to zero due to the formation of a water-insoluble complex. The formation of the interpolymer complex was corroborated by gravimetric and turbidimetric measurements. The dependence of the yield of the complex chitosan/ P($AMPS_{0.1}$-*co*-$AA_{0.9}$) on the mole fraction of aminoglucoside units in mixtures with different ratios of the partners is shown in Fig. 1. The amount of the isolated complex was maximum at a ratio [aminoglucoside units] : [copolymer units] = 0.1 : 0.9. The results on the turbidity of the mixtures (Fig. 2) confirm that, at pH 2.2, a 9-fold excess of the copolymer P($AMPS_{0.1}$-*co*-$AA_{0.9}$) is necessary to achieve formation of the complex with chitosan in maximum amount.

The effect of the pH value of the medium on complex formation was examined in the range from pH 1.5 to 5.8 at constant ionic strength ($I$ = 0.1). Measurements at higher pH values were not performed because of the possibility of precipitation of chitosan at pH > 6. For each P(AMPS-*co*-AA) copolymer, the specific viscosity of the supernatant depended on the ratio [aminoglucoside units] : [copolymer units] and the position of minima depended on the pH of the medium (Figs. 3 - 6). The stoichiometry of the complexes obtained by interaction between chitosan and copolymers with low content of AMPS units was markedly influenced by the pH of the medium (Figs. 3 and 4). On increasing the pH of the medium from 1.2 to 5.8, the minimum of the relationships shifted to a higher fraction of aminoglucoside units, and the complexes were obtained at excess of copolymer.

At low pH values the carboxylic groups of AA are not ionised, thus favouring the formation of ‘loops’ along the copolymer chain (Scheme 1A). In such cases an excess of the copolymer is necessary for the formation of a complex because the ionic interactions proceed only between ionised amino groups of chitosan and sulfo groups of the copolymer. On increasing the pH of the medium, the number of ionised carboxylic groups increases and, hence, the number and the size of ‘loops’ are smaller (Scheme 1B).

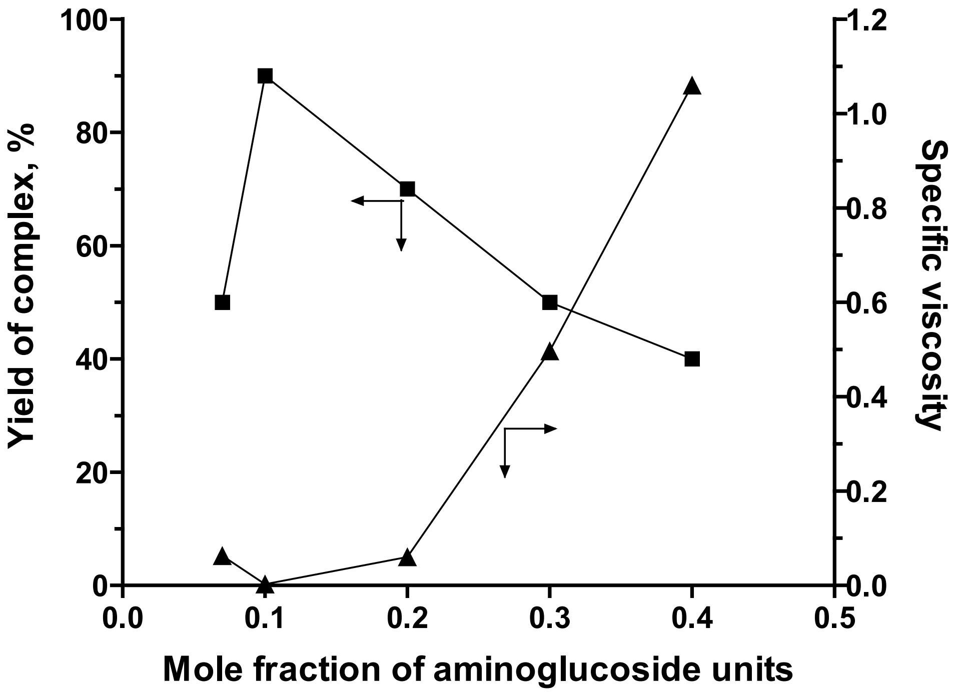



Fig. 1. Dependence of the supernatant specific viscosity (▲) and the yield of complex (■) on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.1}$-*co*-$AA_{0.9}$) (0.2% w/w), pH 2.2, ionic strength *I* = 0.1, 25°C

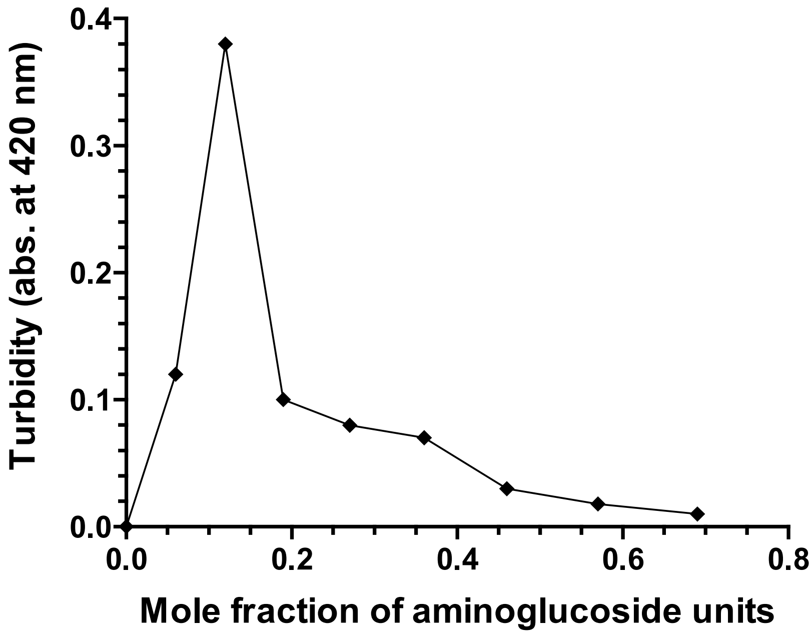



Fig. 2. Dependence of the mixture turbidity on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.1}$-*co*-$AA_{0.9}$) (0.01% w/w), pH 2.2, *I* = 0.1, 25°C

The effect of pH of the medium on the stoichiometry of the complex was less pronounced in the case of copolymers with higher content of AMPS units (Figs. 5 and 6). For the couples chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$) and chitosan/P($AMPS_{0.8}$-*co*-$AA_{0.2}$), the specific viscosity of the supernatant possessed a minimum at a stoichiometry close to the equimolar one.

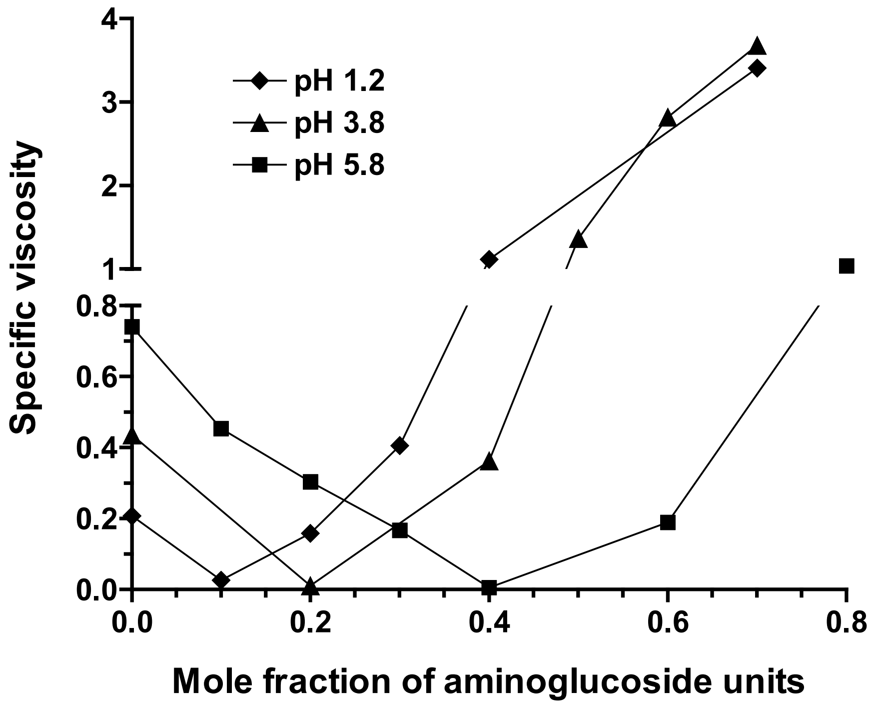



Fig. 3. Dependence of the supernatant specific viscosity on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.1}$-*co*-$AA_{0.9}$) (0.2% w/w) at different pH values, *I* = 0.1, 25°C

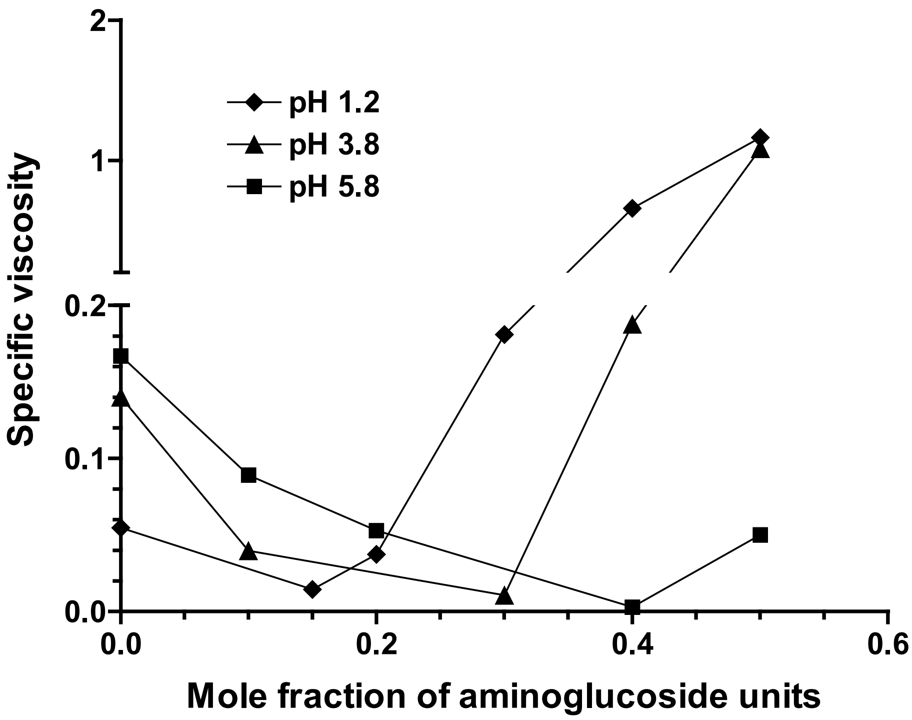
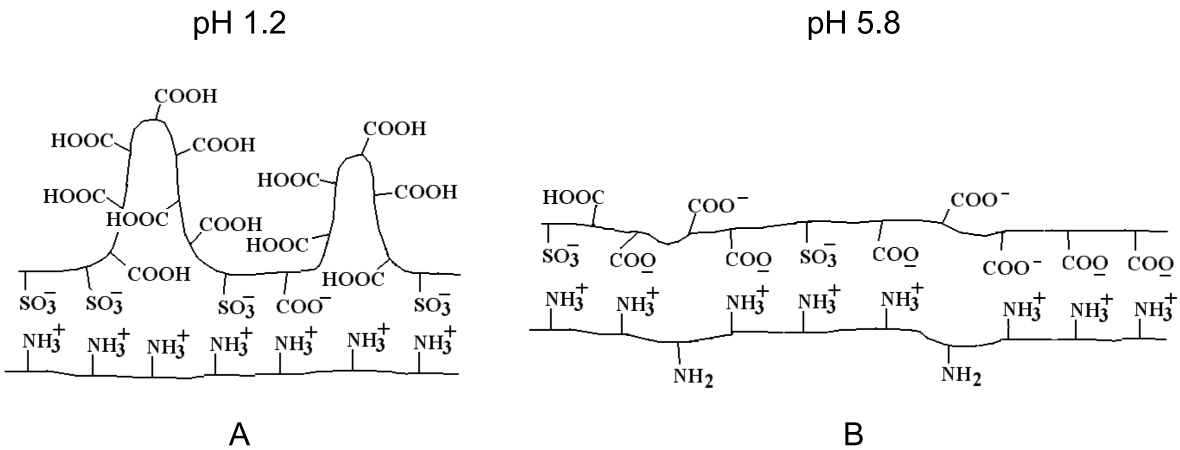



Fig. 4. Dependence of the supernatant specific viscosity on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.2}$-*co*-$AA_{0.8}$) (0.2% w/w) at different pH values, *I* = 0.1, 25°C




Scheme 1. Schematic representation of complex formation between chitosan and copolymers with low content of AMPS units (P[$AMPS_{n'}$-*co*-$AA_n$], n' < n) at different pH of the medium

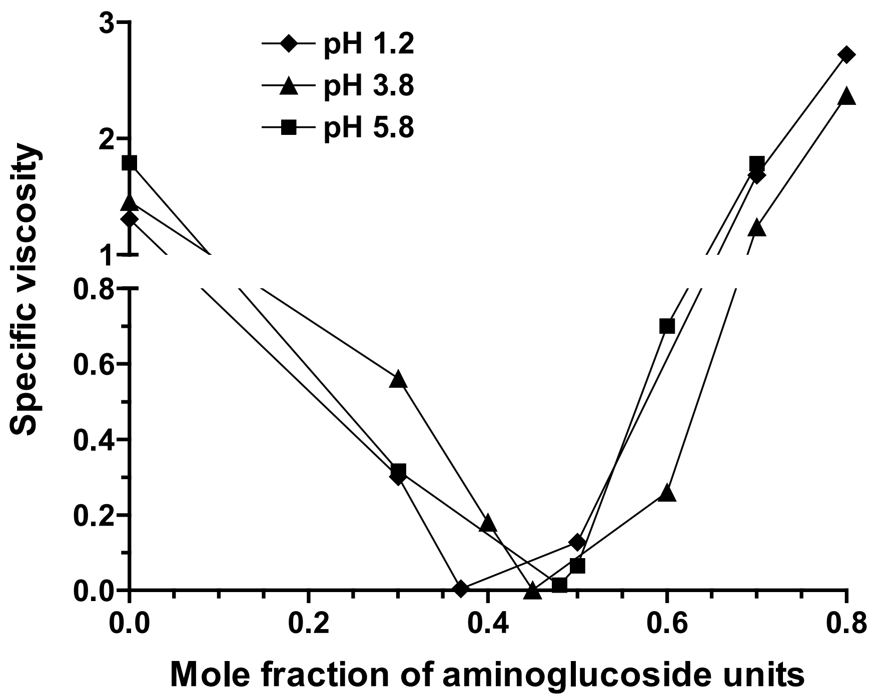



Fig. 5. Dependence of the supernatant specific viscosity on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$) (0.2% w/w) at different pH values, *I* = 0.1, 25°C

The higher the content of AMPS units in the copolymer, the higher the number of complexable $–SO_3H$ groups ionised at each pH value. In this case the formation of 'loops' is negligible not only at higher pH values (pH 5.8) but also at low pH values (pH 1.2) (Scheme 2).

The summarised results from viscometric, gravimetric and turbidimetric measurements on the mole fraction of aminoglucoside units at which the maximum quantity of complex chitosan/P(AMPS-*co*-AA) is obtained ($M_{max}$) are presented in Fig. 7. The obtained results are compared with those for the formation of a complex between chitosan and PAMPS or PAA. While in the case of PAMPS $M_{max}$ was the same in the studied pH range, $M_{max}$ for the copolymers changed with pH. The difference in the behaviour of the copolymers and PAA was more obvious at pH values lower than 3. In contrast to PAA, the copolymers P(AMPS-*co*-AA) having even small amounts of AMPS units form complexes with chitosan in this pH range.

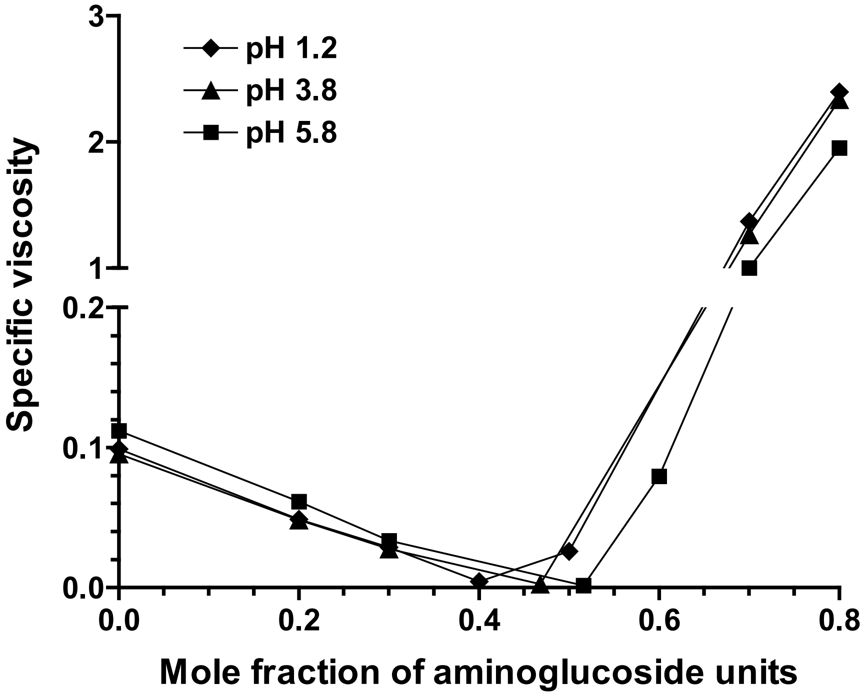
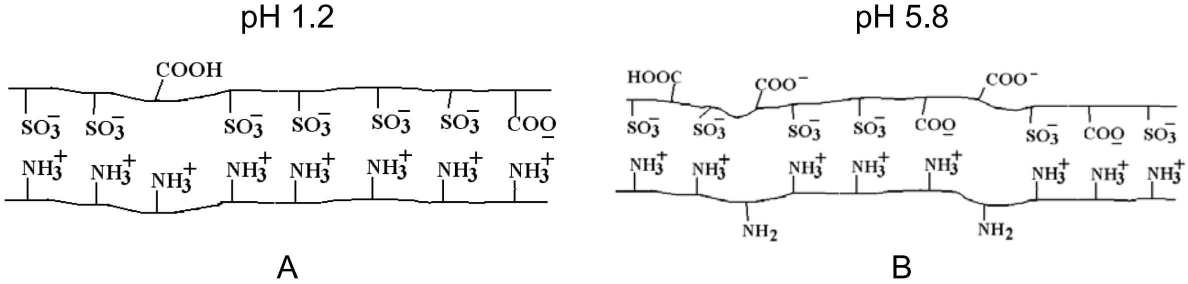



Fig. 6. Dependence of the supernatant specific viscosity on the mole fraction of aminoglucoside units in the feed chitosan/P($AMPS_{0.8}$-*co*-$AA_{0.2}$) (0.2% w/w) at different pH values, $I$ = 0.1, 25°C




Scheme 2. Schematic representation of complex formation between chitosan and copolymers with high content of AMPS units (P[$AMPS_{n'}$-*co*-$AA_n$], n' ≥ n) at two values of pH of the medium

At pH values lower than 4.8 the formation of complexes between chitosan and each of the studied P(AMPS-*co*-AA) copolymers depended strongly on copolymer composition and on the pH of the medium. The value of $M_{max}$ increased on increasing the content of AMPS units in the copolymer and on increasing pH. In the case of P($AMPS_{0.8}$-*co*-$AA_{0.2}$), at pH lower than 4.8 the interaction between chitosan and the copolymer resulted in the formation of PEC with a composition close to the equimolar one. At pH 5.8, due to the presence of a sufficient number of complexable anions along the copolymer chain, $M_{max}$ was affected to a smaller extent by the composition of the copolymers.

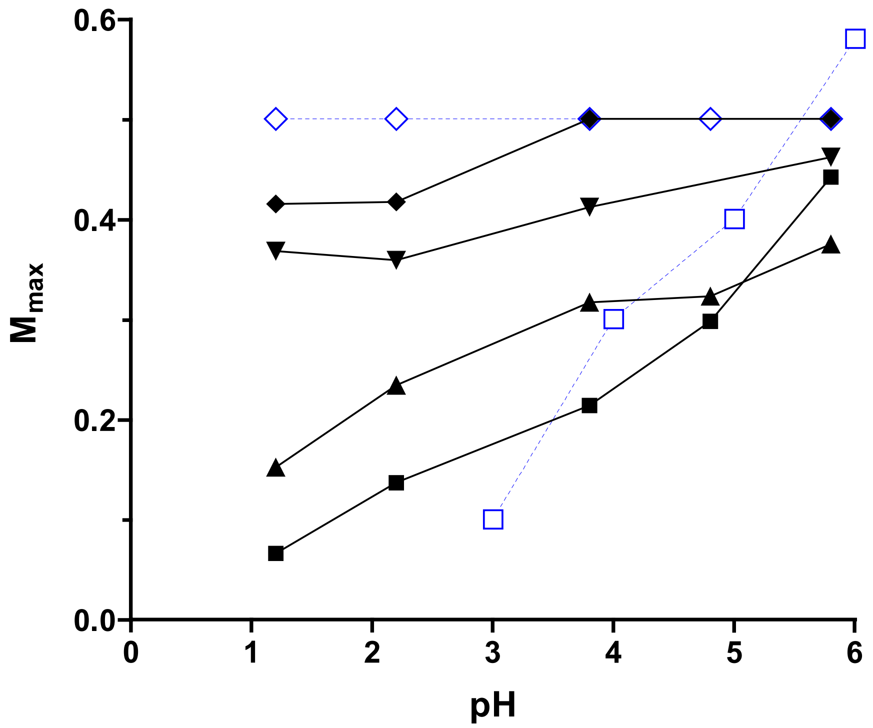



Fig. 7. Dependence of the mole ratio at maximum complex formation, $M_{max}$, on the pH of the medium. (■) Chitosan/P(AMPS$_{0.1}$-*co*-AA$_{0.9}$], (▲) chitosan/P[AMPS$_{0.2}$-*co*-AA$_{0.8}$], (▼) chitosan/P[AMPS$_{0.5}$-*co*-AA$_{0.5}$], (♦) chitosan/P[AMPS$_{0.8}$-*co*-AA$_{0.2}$]. For comparison, $M_{max}$ for the complexes chitosan/PAA (□) calculated from the data of Chavasit et al. [10] and chitosan/PAMPS (◊) [11] are shown

The IR spectra of the complexes differed from those of chitosan and the copolymers by the shift of the bands owing to the sulfo groups towards higher wave numbers (1200 and 1038 $cm^{-1}$). A change in their intensities was also observed: for the complex the stronger band was at 1038 $cm^{-1}$. A broad shoulder appeared at 2500 $cm^{-1}$, which might be assigned to $NH_3^+$ [13]. These findings are indicative of the existence of a strong interaction between chitosan and P(AMPS-*co*-AA) through ionic interactions between amino groups of chitosan and sulfo groups of the copolymer. A characteristic band for the C=O group of AA at 1718 $cm^{-1}$ was observed for the complexes formed at pH values lower than 4.8. This suggested the presence of unionised carboxylic groups along the copolymer chain, which could not interact with ionised amino groups of chitosan.

Complex stability in neutral and alkaline media 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 weight loss was detected. The fractions insoluble at pH 9 and 10 were soluble in acetate buffer solution showing that they consisted of chitosan. Therefore, chitosan/P(AMPS-*co*-AA) complexes are stable up to pH 8.

Previously it was shown that coating chitosan beads with PEC chitosan/PAMPS might be used for sustained drug delivery [14]. The possibility of coating chitosan beads with PEC chitosan/P(AMPS-*co*-AA) was examined. A significant decrease of the diameter of the beads (approx. 2-fold) was observed when copolymers with high content of AMPS units were used (Fig. 8). This collapse was due to complex formation between chitosan and the copolymers at the surface of the beads.

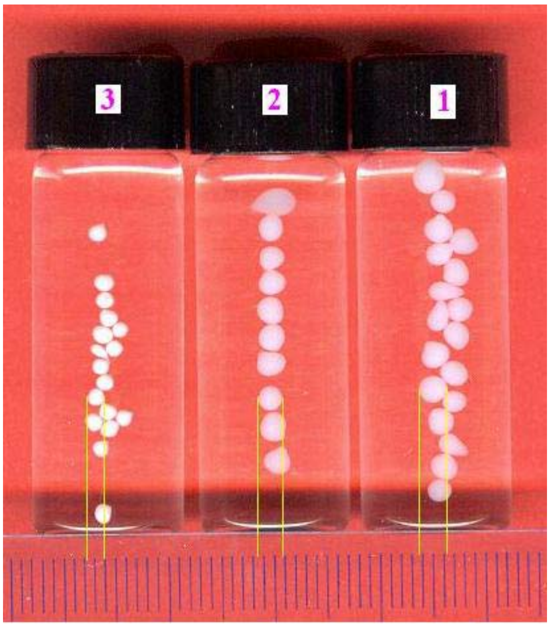
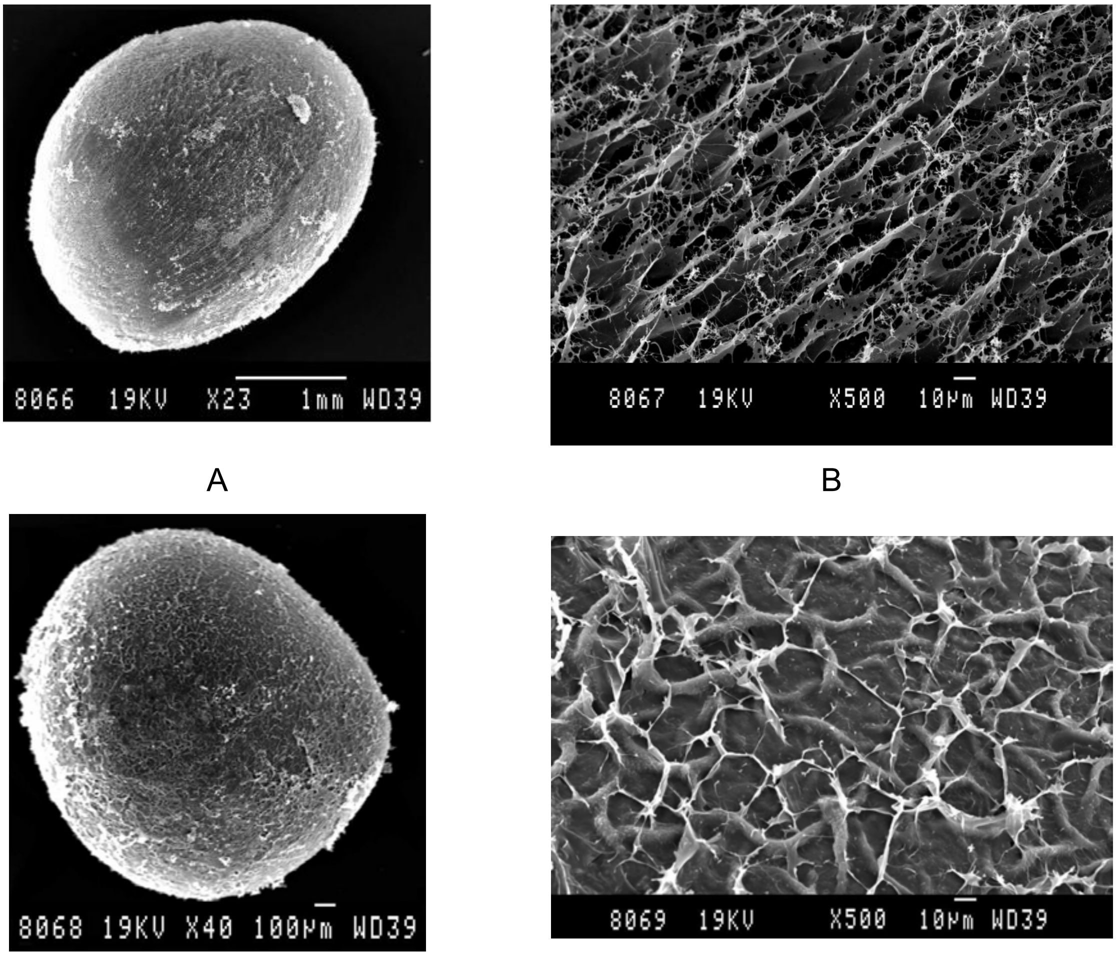



Fig. 8. Chitosan beads, coated with PEC chitosan/P($AMPS_{0.1}$-*co*-$AA_{0.9}$) (1), chitosan/P($AMPS_{0.2}$-*co*-$AA_{0.8}$) (2) and chitosan/P($AMPS_{0.8}$-*co*-$AA_{0.2}$) (3)

Scanning electron micrographs (SEM) of chitosan beads and of chitosan beads, coated with the polyelectrolyte complex chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$) are shown in Fig. 9. The beads were highly porous. After immersing the beads in the solution of P($AMPS_{0.5}$-*co*-$AA_{0.5}$), PEC was obtained at the surface. The surface of the beads treated with copolymer differed from the untreated ones – it was porous, wrinkled and kept the traces of gel collapse.

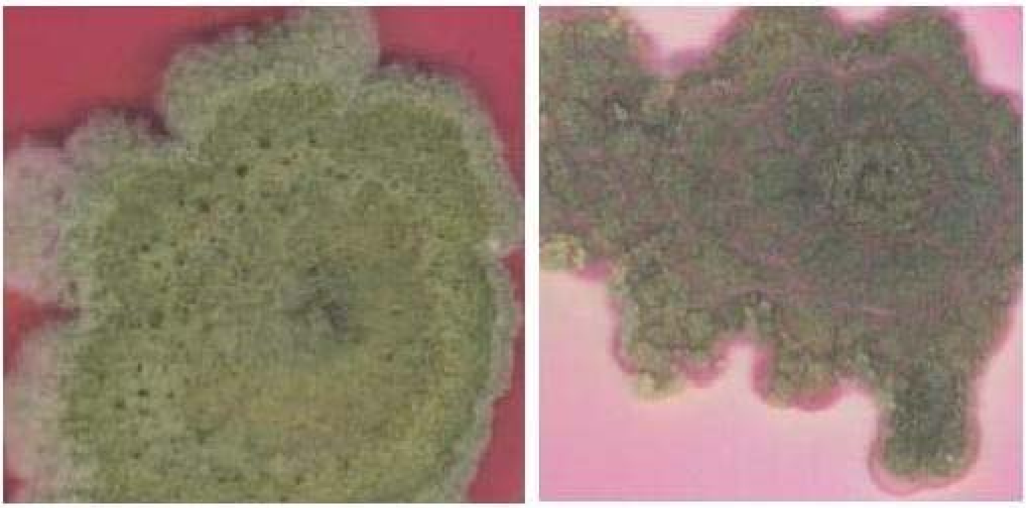
In order to estimate the degradability of chitosan once included in an interpolymer complex, chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$) was treated with crude enzyme complex of the soil fungus *Trichoderma viride* at pH 4. The viscosity-average molecular weight, $\overline{M}_v$, of chitosan decreased from $5.40{\cdot}10^5$ to $1.36{\cdot}10^5$ for 90 min and to $0.99{\cdot}10^5$ for 240 min treatment. Previously it was shown that the values of $\overline{M}_v$ of chitosan from chitosan/PAMPS complex treated under the same conditions were higher (2.08 for 90 min, $1.80{\cdot}10^5$ for 240 min) [15] and those from chitosan/PAA were lower ($1.28{\cdot}10^5$ for 90 min, $0.76{\cdot}10^5$ for 240 min) [16] than the values found in the present work for chitosan/P(AMPS-*co*-AA) complex. It might be assumed that the stability of the complex chitosan/P(AMPS-*co*-AA) increased with increasing content of AMPS units in the copolymers thus leading to more pronounced hydrophobisation of the complexes. The more compact structure and the higher degree of hydrophobisation hamper the enzymatic hydrolysis in the order chitosan/PAA < chitosan/P(AMPS-*co*-AA) < chitosan/PAMPS.

The ability of the obtained complex to be used as a carrier of *T. viride* was investigated. The results obtained by embedding *T. viride* in chitosan beads and in chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$)-coated chitosan beads are shown in Fig. 10. As seen on the photographs hyphae and conidia were formed in both cases showing that *T. viride* developed and reproduced normally. This suggests that the complexes chitosan/P(AMPS-*co*-AA) might be used as appropriate carriers of such microorganisms.




C D

Fig. 9. SEM micrographs of freeze-dried chitosan beads, magnification 23-fold (A) and 500-fold (B); beads treated with P(AMPS$_{0.5}$-*co*-AA$_{0.5}$), magnification 40-fold (C) and 500-fold (D)



A B

Fig. 10. Photographs of *T. viride* embedded in a chitosan bead (A) and in a chitosan/ P(AMPS$_{0.5}$-*co*-AA$_{0.5}$)-coated chitosan bead (B), 4 days after inoculation; 28°C

## Experimental part

### *Materials*

High molecular weight chitosan (molecular weight $6 \cdot 10^5$ given by the supplier, 80% deacetylation), 2-acryloylamido-2-methylpropanesulfonic acid (AMPS) and acrylic acid (AA) were purchased from Fluka. AA was distilled prior to use under reduced pressure in the presence of hydroquinone. The salts used for the redox-initiator system ($K_2S_2O_5$ and $(NH_4)_2S_2O_8$) and those for the preparation of buffer solutions were of analytical grade. The following buffer solutions were used: pH 1.2, 2.2 (HCl/KCl); pH 3.8, 4.8, 5.8 ($CH_3COOH$/NaOH), pH 7 and 8 ($KH_2PO_4$/$Na_2HPO_4$), pH 9.0 and 10.0 ($NaHCO_3$/$Na_2CO_3$).

The culture suspension from *Trichoderma viride* cultivated at 28°C for 3 days in liquid nutrient medium, containing glucose (25 g/l) and corn extract (25 ml/l) was kindly supplied by the Department of Microbial Technologies, Agricultural University of Plovdiv.

### *Preparation of the copolymers*

P($AMPS_{0.1}$-*co*-$AA_{0.9}$) and P($AMPS_{0.2}$-*co*-$AA_{0.8}$) were prepared by free radical copolymerisation of AMPS and AA in aqueous solution according to a procedure described previously [12]. P($AMPS_{0.5}$-*co*-$AA_{0.5}$) and P($AMPS_{0.8}$-*co*-$AA_{0.2}$) were similarly prepared. For example, P($AMPS_{0.5}$-*co*-$AA_{0.5}$) was synthesised by dissolving 2.6 g of AA (1.8 mol/l), 7.5 g of AMPS (1.8 mol/l), and 3.2 ml of 10 M NaOH (1.6 mol/l) in water to a total volume of 20 ml. The reaction mixture was stirred at 20°C. The solution was bubbled with nitrogen for 20 min, then the components of the redox-initiator system, $(NH_4)_2S_2O_8$ (0.0332 g) and $K_2S_2O_5$ (0.0647 g), were added. After 3 h stirring, the copolymer was isolated by precipitation into a large excess of acetone and dried under reduced pressure at 40°C. The copolymer was purified by dialysis against distilled water using a membrane tubing with molecular weight cut-off of 12 000 to 14 000. The conversion was 90% with respect to the total monomer feed.

### *Preparation of the complexes chitosan/P(AMPS-co-AA)*

PECs chitosan/P(AMPS-*co*-AA) were prepared by mixing solutions of chitosan and P(AMPS-*co*-AA) in buffers with different pH: 1.2, 2.2, 3.8, 4.8 and 5.8, and constant ionic strength ($I$ = 0.1). For performing gravimetric and viscometric measurements, PECs were prepared by mixing 0.2% solutions of chitosan and P(AMPS-*co*-AA). Reactant solutions with equal pH values were mixed in the following volumetric proportions (chitosan : P(AMPS-*co*-AA)): 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. The obtained white precipitate was isolated by centrifugation (40 min, 4000 rpm), washed three times with deionized water and dried to constant weight. The yield of the obtained complexes was evaluated gravimetically.

In the case of turbidimetric measurements, PECs were prepared by mixing 0.01% solutions of chitosan and P(AMPS-*co*-AA). The turbidity of the mixtures (absorbance at 420 nm) was registered on a UV-VIS SPECORD 71 spectrophotometer.

The obtained results (yield of complex, viscosity of the supernatant solutions and turbidity) are presented as a function of the mole fraction of the aminoglucoside units in the feed: $M = A / (A+B)$, where $A$ represents the amount of aminoglucoside units and $B$ of (AMPS-*co*-AA) units, respectively.

For determination of the stability of the complex, samples of the complex chitosan/ P(AMPS-*co*-AA) were stirred for 100 h at 25°C in buffer solutions. The mixtures were centrifuged (40 min, 4000 rpm), and the isolated precipitates were washed with deionized water and dried to constant weight.

### *Methods for analysis and characterisation*

The molecular-weight characteristics ($\overline{M}_n$, $\overline{M}_v$, $\overline{M}_w$, $\overline{M}_w/\overline{M}_n$) of the copolymers were determined by size-exclusion chromatography (SEC) on a Waters apparatus equipped with a set of 3 Shodex OH pak (SB 806M) columns, differential refractometer and viscometer detector. The eluent was 0.5 M $LiNO_3$ with $6 \cdot 10^{-3}$ M $NaN_3$ at a flow rate of 0.75 ml/min at 40°C. Pullulan (PSL, MW $22.8 \cdot 10^3$ - $788 \cdot 10^3$) and polyoxyethylenes (Waters, MW $10.8 \cdot 10^3$ - $690 \cdot 10^3$) were used as standards for calibration.

Elemental analysis for carbon, hydrogen, nitrogen and sulfur was conducted for estimation of the composition of the copolymers.

IR spectra of P(AMPS-*co*-AA), chitosan and their complexes were registered on a spectrophotometer FT-IR Bruker Vector 22 using the KBr pellet technique.

$^{13}C$ NMR spectra of copolymers were taken on a Bruker MSL 300 spectrometer operating at 75.5 MHz at 300 K in $D_2O$, using tetramethylsilane as a reference.

The viscosity of supernatant solutions was measured with an Ubbelohde viscometer at 25 ± 0.1°C.

### *Preparation of chitosan/P(AMPS-co-AA)-coated chitosan beads*

Chitosan beads were prepared by capillary extrusion of chitosan solution (1.5% in 0.5% aqueous solution of $CH_3COOH$) in 5% aqueous solution of NaOH. The obtained beads were kept in the alkaline solution for 24 h and then washed with distilled water until the aqueous phase was neutral. Chitosan/P(AMPS-*co*-AA)-coated chitosan beads were prepared by immersing the beads in 3% aqueous solution of P(AMPS-*co*-AA) for 2 h followed by washing with distilled water until the aqueous phase was neutral. The surface morphology of the beads was studied with a scanning electron microscope JEOL JSM - 840 A. Specimens were placed on the sample holders with a double-sided adhesive tape, vacuum-coated with platinum film and then observed.

### *Degradation of chitosan from the complex chitosan/P(AMPS-co-AA)*

The degradation study was performed using a crude enzyme mixture produced by the soil fungus *Trichoderma viride* with an activity of 0.0038 U/ml for 90 and 240 min as described earlier [16].

### *Microbiological tests*

Beads of chitosan containing 50 mg biomass of *T. viride* per 1 g polymer were prepared. A portion of the beads was coated with PEC chitosan/P($AMPS_{0.5}$-*co*-$AA_{0.5}$).

The ability of the embedded microorganisms to develop was tested by inoculation of the beads on the surface of Rose-Bengal chloramphenicol agar (Oxoid) at 28°C.

*Acknowledgement:* Financial support from the Bulgarian Ministry of Education and Science (Contract CH-1105) is gratefully acknowledged. The authors thank M. Naydenov from the Laboratory of Microbiological Technologies at the Agricultural University of Plovdiv for the tests.

[1] Dumitriu, S; Chornet, E.; *Adv. Drug Deliv. Rev.* **1998**, *31*, 223.

[2] Lee, K.; Park, W.; Ha, W.; *J. Appl. Polym. Sci.* **1997**, *63*, 425.

[3] Anderson, M.; Löfroth, J.; *Int. J. Pharm*. **2003**, *257*, 305.

[4] Skorikova, E.; Kalyuzhnaya, R.; Vikhoreva, G.; Gal'braikh, L.; Kotova, S.; Ageev, E.; Zezin, A.; Kabanov, V.; *Vysokomol. Soedin., Ser. A* **1996**, *38*, 61.

[5] Peniche, C.; Argüelles-Monal, W.; *Macromol. Symp*. **2001**, *168*, 103.

[6] Takahashi, T.; Takayama, K.; Machida, Y.; Nagai, T.; *Int. J. Pharm*. **1990**, *61*, 35.

[7] Mireles, C.; Martino M.; Bouzas, J.; Torres, J.; in "*Advances in Chitin and Chitosan*", Brine, C.; Sandford, P.; Zikakis, J., editors; Princeton **1991**, p. 506.

[8] Skorikova, E.; Vikhoreva, G.; Kalyuzhnaya, R.; Zezin, A.; Gal'braikh, L.; Kabanov, V.; *Vysokomol. Soedin., Ser. A* **1988**, *30*, 44.

[9] Cerrai, P.; Guerra, G.; Tricoli, M.; Maltini, S.; Barbani, N.; Petarca, L.; *Macromol. Chem. Phys.* **1996**, *197*, 3567.

[10] Chavasit, V.; Kienzle-Sterzer, C.; Antonio Torres, J.; *Polym. Bull*. **1988**, *19*, 223.

[11] Stoilova, O.; Koseva, N.; Manolova, N.; Rashkov, I.; *Polym. Bull.* **1999**, *43*, 67.

[12] Iliopoulos, I; Auderbert, R.; *Macromolecules* **1991**, *24*, 2566.

[13] Takayama, K.; Hirata, M.; Machida, Y.; Masada, T.; Sannan, T.; Nagai, T.; *Chem. Pharm. Bull.* **1990**, *38*, 1993.

[14] Koseva, N.; Stoilova, O.; Manolova, N.; Rashkov, I.; *J. Bioact. Compat. Polym.* **2000**, *15*, 1.

[15] Stoilova, O.; Koseva, N; Petrova, T., Manolova, N.; Rashkov, I.; Naydenov, M.; *J. Bioact. Compat. Polym.* **2001**, *16*, 379.

[16] Paneva, D.; Mincheva, R.; Stoilova, O.; Manolova, N.; Rashkov, I.; *e-Polymers* **2003**, no. 019.