

Chitosan–Cellulose Sulfate Acetate Complexation in Acetic Acid Solutions

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Received August 8, 2007

Abstract—Colloidal and optical properties of dispersions of chitosan–cellulose sulfate acetate interpolyelectrolyte complexes resultant from mixing dilute solutions of the polymers in acetic acid and in acetic acid–based buffer mixtures are investigated. It is established that, in acetic acid, an insoluble complex is formed whose composition corresponds to the unit ratio [chitosan] : [cellulose sulfate acetate] = 1 : 1.5, mol/mol. Particle size and concentration are independent of the order of mixing of the solutions of the polymers. In buffer solvents, the particle size is larger and the particle concentration is lower than those in acetic acid. Excess chitosan causes the dissolution of the complex. The addition of low-molecular-weight electrolytes to ionic strengths of 0.2–0.3 also promotes the dissolution of the interpolyelectrolyte complex. The complex becomes completely soluble at ionic strengths of 1.5–2.0.

DOI: 10.1134/S1061933X08050165

INTRODUCTION

In dilute aqueous solutions of acids, chitosan is known to interact with various polymers (hyaluronic acid [1], alginates [2], pectin, dextrin, carrageenan, carboxymethyl cellulose, poly(acrylic acid) [3], poly(vinyl alcohol) [4], poly(ϵ -caprolactam) [5], etc.). As a result, interpolymer complexes are formed, which are used in medicine, genetic engineering, membrane separation of liquid media, desalination, purification of water, etc. [6].

We obtained interpolyelectrolyte complexes (IPECs) based on chitosan and a mixed ester of cellulose, that is, cellulose sulfate acetate (CSA) [7]. Depending on the concentration, order and mixing procedure of solutions of polymers, as well as on their chemical composition and the nature of solvents, IPECs may be prepared in the forms of microcapsules, coacervates, gels, films, fibrous materials, particles with different sizes and shapes, etc.

The goal of this work is to study the processes and the products of chitosan–CSA interaction in acetic acid and its buffer solutions and, in particular, to determine the influence of solvent composition and the order of mixing of dilute polyelectrolyte solutions on the concentration, size, composition, and solubility of resultant IPEC particles.

EXPERIMENTAL

Chitosan produced from crabs in ZAO Bioprogress with the degree of acetylation equal to 0.24 ± 0.03 and a viscosity-average molecular mass of $M_{\eta} = 3.09 \times 10^5$

was applied in this work. In aqueous solutions of acids, chitosan demonstrates the properties of a cationic polyelectrolyte ($pK = 6.5$ [8]). CSA with $M_{\eta} = 4.6 \times 10^4$ and the degree of substitution with respect to acetate and sulfate groups equal to 1.5 ($pK = 2.9$) synthesized by the homogeneous method was used as an anionic polyelectrolyte.

Acetic acid concentration in the solutions was 0.16 M, and sodium acetate concentrations in the buffer solutions were equal to 0.1, 0.2, and 0.3 M.

Interaction of the polyelectrolytes was investigated by the colloidal titration method based on the ability of the system to scatter light when a dispersion of insoluble particles is formed. In order to measure the optical density of formed IPEC dispersions, a quartz cell containing a 0.001 M solution of one of the polymers (20 cm^3) was dropwise fed with a 0.01 M solution of another polymer (titrant) under vigorous stirring. (Polymer concentrations are expressed in moles of monomer units. In the case of chitosan, the average molecular masses of chitin and chitosan units are applied with allowance for their molar fractions in the polymer.) The aforementioned titrant concentration was selected with allowance for the minimum dilution in the course of titration. The concentration of a titrated polymer solution ensured the formation of a finely dispersed IPEC suspension, which was stable for more than 1 h, i.e., for a period longer than the time of titration. Titration was carried out under continuous stirring at a rate of titrant addition equal to 0.1 ml/min and a temperature of $20 \pm 2^\circ\text{C}$. CSA solutions were titrated

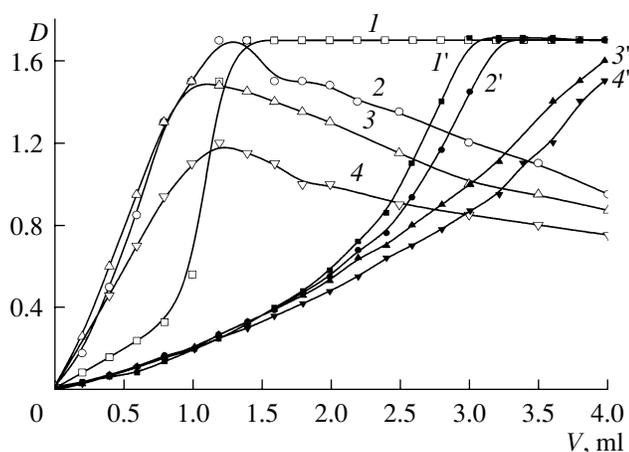


Fig. 1. Titration curves of (1–4) a CSA solution with a chitosan solution and (1'–4') a chitosan solution with a CSA solution in (1, 1') 0.16 M acetic acid and its buffer solutions of the following compositions: (2, 2') 0.16 M CH_3COOH + 0.1 M CH_3COONa , (3, 3') 0.16 M CH_3COOH + 0.2 M CH_3COONa , and (4, 4') 0.16 M CH_3COOH + 0.3 M CH_3COONa .

with chitosan solutions and chitosan solutions were titrated with CSA solutions.

Optical density D was measured 1 min after the addition of each portion of titrant using KFK-2-UKhL I.2 and KFK-3-01 photoelectrocolorimeters. In a series of parallel experiments, differences in D values were no higher than the measurement errors of the instruments. As the initial polyelectrolytes and formed IPECs have no absorption bands in the visible range, the measurements were performed at the wavelength of incident light $\lambda = 440$ nm. The D – λ dependence, which was necessary for the determination of the size and concentration of dispersed phase particles by the turbidity spectrum method [9], was registered with a SPECORD-132M spectrometer in a range of 200–700 nm. This dependence appeared to only be linear in a range of 330–420 nm. It was this spectrum range in which the values of \bar{r}_λ and N were calculated via the following equations:

$$\bar{r}_\lambda = \frac{\alpha \lambda_{\text{av}}}{2\pi\mu_0}; \quad N = 12.6 \frac{\tau_{\text{av}} \mu_0^2}{\lambda_{\text{av}}^2 K(\alpha, m) \alpha^2}.$$

Here, \bar{r}_λ is the average effective radius of dispersed particles; λ_{av} is the middle of the wavelength range of the linear part of the $\log\lambda$ – $\log D$ plot; μ and μ_0 are the refractive indices of dispersed phase particles and dispersion media, respectively; m is the relative refractive index of the particles; α is the particle size-to-light wavelength ratio or the relative size of a particle; τ_{av} is the turbidity of the system at λ_{cp} ($\tau = 2.3D/l$, where l is the light path); K is the efficiency factor tabulated as a

function of α at different m values; and N is the number of particles in 1 cm^3 of a dispersion.

In spectral experiments, dye [methylene blue (MB)] concentration amounted to 0.025 g/dm^3 .

Micrographs of IPEC particles were taken with a LEO-1420 scanning electron microscope. Samples were prepared through the application of suspensions (1 cm^3) onto microscope slides with sizes of 1.5×1.5 cm^2 followed by drying in air and deposition of gold onto their surface (a K550 EMITECH instrument was employed).

RESULTS AND DISCUSSION

As is seen from Fig. 1 (curves 1, 1'), during the titration of chitosan or CSA solutions in acetic acid, the optical density increases and the titration curves reach plateaus irrespective of which polymer solution is used as a titrant. The increase in the optical density is caused by chitosan–CSA interaction yielding suspensions in which insoluble IPEC particles represent a dispersed phase, while the dispersion media may contain both the solvent and macromolecules of a polymer that is present in excess. The ratios of the polyelectrolytes, at which the titration curves reach plateaus, characterize the composition of IPECs. The formation of insoluble IPECs is related to mutual screening of the charges of interacting macromolecules and transformation of hydrophilic polymers into hydrophobic products [10]. Protonated amino groups of chitosan and ionized sulfate groups of CSA are involved in the electrostatic interaction. Unfortunately, the fraction of the groups involved in the intermolecular bonding appeared to be difficult to quantitatively determine by the traditional methods including potentiometric or conductometric titration because of the buffering properties of the solvents and an insubstantial contribution of high-molecular-weight cations and anions to the conductivity of the system in the examined concentration range [11].

By the example of the titration of a CSA solution with a chitosan solution, it was shown that, by the moment when the colloidal titration curve (CTC) reaches the plateau, the polymer that had initially been taken in excess was completely involved in the interaction. For example, Fig. 2 shows the absorption spectra of MB in acetic acid and in the systems whose compositions correspond to different points in CTCs. The application of MB as an indicator of free CSA was based on its metachromatic interaction with this polymer [12], which is accompanied by a passage from the blue to the violet color of the solutions (curve 3). MB does not interact with chitosan.

As follows from Fig. 2, at initial stages of the titration, e.g., at a ratio of [chitosan] : [CSA] = 1 : 4, mol/mol, CSA is not entirely complexed. This is evident from the metachromatic absorption band of MB in the region of 560 nm (Fig. 2, curve 4). At a ratio of [chitosan] : [CSA] = 1 : 1.5, mol/mol, which corresponds to

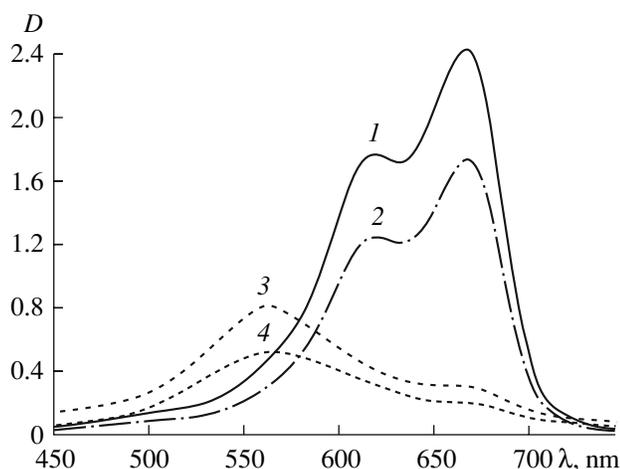


Fig. 2. Absorption spectra of MB (1) in acetic acid, (3) in a CSA solution in acetic acid, and (2, 4) in dispersions with compositions of [chitosan] : [CSA] = (3) 1 : 1.5 and (4) 1 : 4.

reaching the plateau, the spectrum of individual MB is recorded (curve 2).

According to the data in Fig. 1 (curves 1, 1'), the molar ratio between the polymers composing insoluble IPEC is 1 : 1.5. It should however be noted that the composition of the complexes depends on the ratio between sulfate groups occurring in H^+ and Na^+ forms in macromolecules of CSA. The amount of these groups is judged from pH of an aqueous 1% polymer solution. The CSA sample used in this work (pH 1.5) predominantly contained these groups in H^+ form. Their dissociation in a 0.16 M acetic acid solution is inhibited. As the protonated amino groups of chitosan mainly interact with ionized sulfate groups occurring in the salt form, the complex is enriched with CSA. The dependences of the complex composition on the contents of sulfate groups occurring in the acid and salt forms in CSA samples are illustrated in Fig. 3.

As follows from Fig. 3, the lower the content of sulfate groups occurring in the H^+ form in a sample (that is, the higher pH of its 1% aqueous solution), the closer the chitosan : CSA ratio is to 1 : 1.

According to the scanning electron microscopy data, the solid phase of an IPEC dispersion has the form of aggregates composed of compact particles or plates with different sizes (Fig. 4).

When sodium acetate is added to acetic acid, i.e., upon the passage to the buffer solutions (Fig. 1, curves 2–4 and 2'–4'), the pattern of CTCs changes. For example, when a CSA solution is titrated with a chitosan solution, a region of a decrease in D appears rather than the plateau, this decrease becoming more intense with an increase in the content of sodium acetate. Moreover, in the case of the buffer solvents, the chitosan : CSA ratio becomes equal to 1 : 2 and is almost independent of the salt content. Most likely, in the presence of the salt, on one hand, the degree of ionization

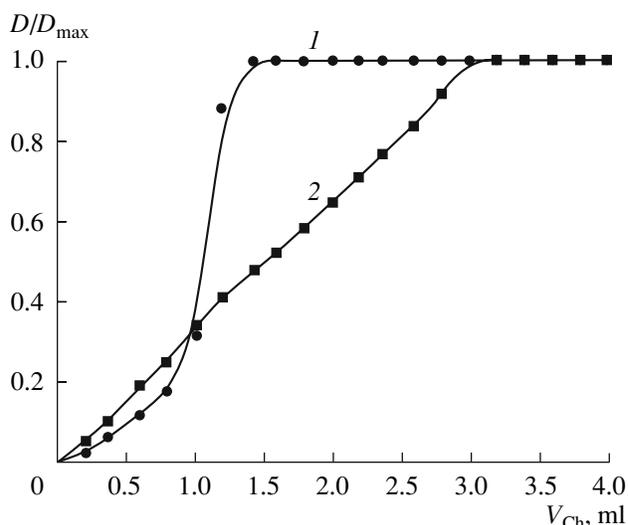


Fig. 3. Titration curves of CSA solutions with pH (1) 1.5 and (2) 3.89 with chitosan solution.

of CSA macromolecules rises due to the inhibited dissociation of acetic acid and, on the other hand, ionized sulfate groups are screened owing to the increased concentration of low-molecular-weight ions. As a result, the effective charges of the chains decrease and, as a consequence, the amount of CSA in IPEC rises. As the sodium acetate concentration is further increased, the concentration of complex particles declines that is evident from a decrease in the CTC plateau height. This decline in the concentration of dispersed phase particles was also confirmed by the turbidity spectrum method (Fig. 5).

Moreover, when passing from acetic acid to its buffer solutions, the parameter of the linear charge density on chitosan chains decreases from 1.02 to 0.95, while the average intercharge distance rises from 7 to 7.3 [13]. This decrease in the charge density on titrant macromolecules results in a decline in the number of particles in a formed IPEC dispersion. In addition to a reduction in the IPEC particle concentration, their sizes are enlarged in the buffer solutions. Moreover, particle radii are independent of the polymer ratios (Fig. 5, curves 1', 2').

The effect of the salts is also evident from the finding that, when CSA is titrated with a chitosan solution, CTC demonstrates maximum rather than plateaus, as in the case of acetic acid (Fig. 1, curves 2–4). According to [14], this fact may be explained by the partial dissolution of the complexes. The dissolution of IPEC is caused by the incorporation of large numbers of ionized units into the particles, with these units being uninvolved in the formation of interchain salt bonds. In this case, these units belong to chitosan, which is incorporated into IPEC in excess, has a higher molecular mass than CSA, and plays the role of a lyophilizing component [14].

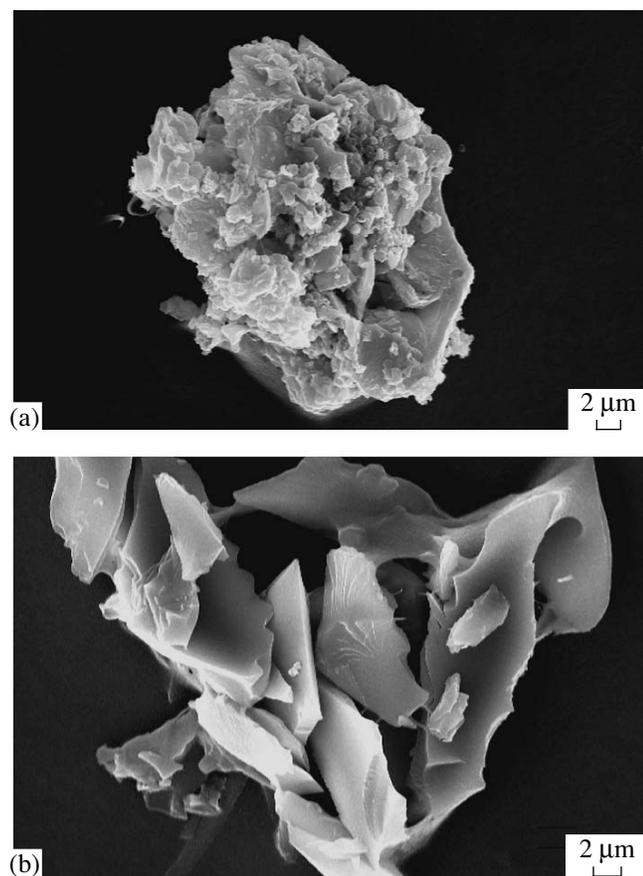


Fig. 4. IPEC particles resultant from the addition of (a) a chitosan solution to a CSA solution and (b) CSA solution to a chitosan solution.

At the same time, the addition of excess CSA, which is a blocking short-chain polymer, to an insoluble IPEC does not make the latter soluble. Soluble IPECs are formed in the presence of excess chitosan only in buffer solutions rather than in acetic acid. This is likely due to the fact that, in salt solutions, CSA macromolecules involved in the interaction occur in a more unfolded conformation, and the structure of the formed insoluble IPEC particles appears to be looser. This structure provides intact sulfate groups with a higher accessibility to the interaction with chitosan amino groups. The fact that the dissolution of IPEC is not accompanied by its disintegration into the initial components is confirmed by the blue rather than the violet color of the dispersion medium after MB is added. The absence of a violet color indicates that free CSA molecules have not appeared in the system.

The IPEC dissolution, which is evident from a decrease in the optical density, may also take place when a low-molecular-weight salt alone is added to the system. The behavior of an IPEC dispersion in acetic acid upon the addition of 4 M solutions of NaCl, NaNO₃, AcOK, AcONH₄, and AcONa is illustrated in Fig. 6, where CTCs are presented as the dependences of the optical density normalized relative to its maximum value on solution ionic strength. It appears that, when the salts are added to a system containing predominantly insoluble IPEC ([chitosan] : [CSA] = 1 : 2) (Fig. 6), the optical density initially increases, thus suggesting a rearrangement of complex particles; furthermore, it declines, thus indicating the transformation of the insoluble IPEC into a soluble one. When the ionic strength is substantially enhanced (to 1.5–2.0), the complete dissolution is achieved.

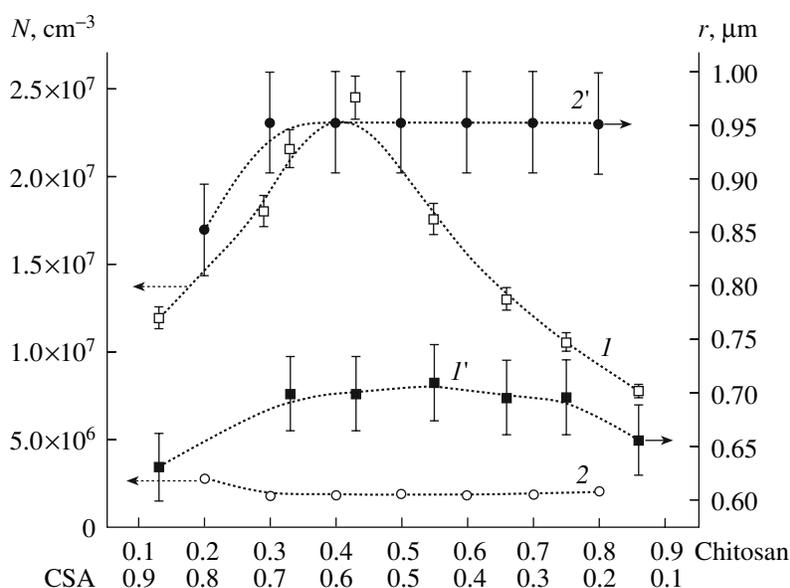


Fig. 5. Dependences of (I , 2) concentration and (I' , 2') radius of IPEC particles on the molar composition of the initial mixture of the polymers in (I , I') 0.16 M acetic acid and (2 , $2'$) a 0.16 M CH₃COOH + 0.1 M CH₃COONa buffer solution.

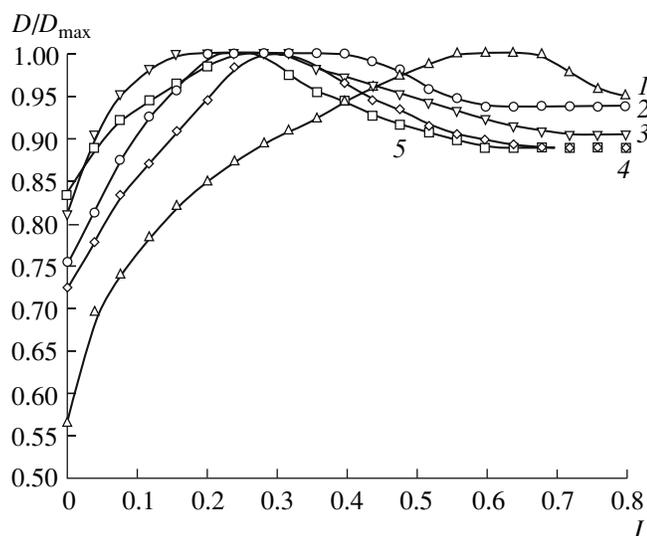


Fig. 6. Dependences of the optical density of an IPEC dispersion ($[\text{chitosan}] : [\text{CSA}] = 1 : 2$) on the ionic strength of (1) AcOK, (2) AcONa, (3) NaCl, (4) NaNO_3 , and (5) AcONH_4 solutions.

As is seen from Fig. 6, the composition of the salts insubstantially influences the shift of the equilibrium toward soluble IPECs. In all cases, except for AcOK, the complex dissolution is observed when the ionic strength becomes higher than 0.2–0.3. The IPEC dissolution may be related to the dissociation of some salt bonds between the units of the lyophilizing and blocking polymers in the presence of a low-molecular-weight electrolyte [15].

CONCLUSIONS

The interaction between dilute solutions of chitosan and CSA in acetic acid gives rise to the formation of an insoluble IPEC as a new phase. Its composition depends on the H^+ - to- Na^+ form ratio of sulfate groups in a CSA sample and may be varied over a range of $[\text{chitosan}] : [\text{CSA}] = 1 : 1.5$ – $1 : 1$. The lower the content of sulfate groups occurring in the H^+ form in CSA, the closer the ratio between the complexed polymers to 1 : 1. Formed IPECs become soluble in the presence of low-molecular-weight electrolytes when a solution ionic strength of 0.2–0.6 is reached. For the complete dissolution of IPECs, the ionic strength must be no less than 1.5–2.0.

The passage from acetic acid to its buffer solutions leads to a reduction in the number of formed IPEC particles and a rise in their size, which is independent of the ratio between the polymers in both solvents. In the

presence of excess chitosan, both insoluble and soluble IPECs are formed in buffer solutions.

REFERENCES

- Chen, J., Li, Q., Xu, J., et al., *Artif. Organs*, 2005, vol. 29, p. 104.
- Chandy, T., Mooradian, D.L., and Rao, G.H.R., *Artif. Organs*, 1999, vol. 23, p. 894.
- Ageev, E.P., Kotova, S.L., Skorikova, E.E., and Zezin, A.B., *Vysokomol. Soedin., Ser. A*, 1996, vol. 38, p. 323.
- Duan, B., Yuan, X., Zhu, Y., et al., *Eur. Polym. J.*, 2006, vol. 42, p. 2013.
- Liar, S.-K., Hung, C.-C., and Lin, M.-F., *Macromol. Res.*, 2004, vol. 12, p. 466.
- Il'ina, A.V. and Varlamov, V.P., *Prikl. Biokhim. Mikrobiol.*, 2005, vol. 41, p. 9.
- Shibailo, T.N., Abstracts of Papers, *Mezhdunarodnaya konferentsiya molodykh uchenykh po fundamental'nykh naukam "Lomonosov-2006"* (Int. Conf. of Young Scientists on Fundamental Sciences "Lomonosov-2006"), (Moscow, 2006), Moscow: Khimiya, 2006, p. 203.
- Gamzazade, A.N., in *Khitin i khitozan: poluchenie, svoistva i primeneniye* (Chitin and Chitosan: Synthesis, Properties, and Applications), Skryabin, K.G., Vikhoreva, G.A., and Varlamov, V.P., Eds., Moscow: Nauka, 2002, p. 112.
- Klenin, V.I., Shchegolev, S.Yu., and Lavrushin, V.I., *Kharakteristicheskie funktsii svetorasseyaniya dispersnykh sistem* (Characteristic Functions of Light Scattering by Disperse Systems), Saratov: Saratovsk. Gos. Univ., 1977.
- Kharenko, O.A., *Vysokomol. Soedin., Ser. A*, 1979, vol. 21, p. 2719.
- Bobreshova, O.V., Bobylkina, O.V., Kulintsov, P.I., et al., *Elektrokhimiya*, 2004, vol. 40, p. 793.
- Sergei, E.V., Abstracts of Papers, *X Respublikanskaya nauchnaya konferentsiya studentov i aspirantov vuzov Respubliki Belarus' "NIRS-2005"* (X Scientific Conf. of Students and Post-Graduated Students of Belarus Republic Institutes "NIRS-2005"), Minsk, 2005, part 2, p. 288.
- Safronov, A.P., in *Khitin i khitozan: poluchenie, svoistva i primeneniye* (Chitin and Chitosan: Synthesis, Properties, and Applications), Skryabin, K.G., Vikhoreva, G.A., and Varlamov, V.P., Eds., Moscow: Nauka, 2002, p. 130.
- Zeizin, A.B. and Kabanov, V.A., *Usp. Khim.*, 1982, vol. 51, p. 1447.
- Chupyatov, A.M., Rogacheva, V.B., Zezin, A.B., and Kabanov, V.A., *Vysokomol. Soedin., Ser. A*, 1994, vol. 36, p. 212.