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BRIEF COMMUNICATIONS

A Study of the Interaction of Chitosan with Cefazolin

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Abstract—Some aspects of the complexation of chitosan with an antibiotic cefazolin were studied. The optimal conditions for synthesis of the complexes were found. Their composition and physicochemical properties were analyzed.

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Chitosan, a polyaminosaccharide of natural origin, possesses a number of unique properties, owing to which it finds wide use in medicine. The solubility of chitosan in weakly acid aqueous solutions, its film-forming capacity, and presence of primary amino groups in the composition of this polysaccharide enable its use for fabrication of membranes and film coatings containing immobilized medicinal compounds [1–6]. Recently, a considerable researchers' attention has been given to analysis of complexes formed by chitosan and medicinal substances [7, 8]. The reason is that the biopolymer not only prolongs the action of medicinal agents, diminishes their toxicity, and enhances the curing effect, but also provides efficient supply of preparations to target organs.

The aim of the study was to analyze the interaction of chitosan (CTS) with a derivative of cefalosporinic acid, cefazolin (CFZ), which exhibits an antimicrobial effect [9], and to obtain films with controllable release of the medicinal agent on their basis.

EXPERIMENTAL

The CTS used in the study was produced from shells of the Far-Eastern crab and had the following parameters: initial number-average degree of polymerization Pn 680, degree of deacetylation 70–75%, and moisture content 4.5%. A pharmaceutical preparation of CFZ was used without additional purification.

IR spectra of the samples were recorded with Specord-M 80 and Shimadzu spectrometers (KBr pellets, films) in the range 700–3600 cm^{-1}. UV spectra of all the samples were recorded relative to water in 1-cm-thick quartz cuvettes with a Specord M-40 spectrophotometer in the range 220–350 nm. As solvent served 0.1, 1, 10, and 70% acetic acid (AA). ^1H NMR spectra were recorded with a Bruker AM-300 instrument (working frequency 300 MHz, solvent D_2O). The pH value of the solutions was monitored with an ANION 4100 pH-meter.

The interaction products were obtained by mixing cefazolin and chitosan in acetic acid of various concentrations (1, 10, 70%) at $T = 25°C$ in the course of 1 h. The compounds synthesized were purified by double reprecipitation of the reaction solution into isopropanol (at a 1 : 5 volume ratio of the reaction mixture and isopropanol), followed by washing of the precipitated complex with isopropanol. Further, the precipitate was dried in a vacuum to constant weight.

Films were produced from a 2% solution of the CTS–CFZ complex, with a 1 : 1 molar ratio of the components, in 1, 10, and 70% acetic acid by casting onto glass, with the subsequent drying in air at room temperature. The thickness of the films was the same (0.1 mm) in all the experiments. The kinetics of CFZ release from film samples placed in an aqueous solution was studied photometrically with a Specord M-40 on the basis of the absorption band of CFZ at $\lambda = 270$ nm. To preclude dissolution in water, chitosan films were preliminarily treated with a 5% solution of an anionic surfactant, sodium dodecyl sulfate. The time of keeping the films in the surfactant solution was 10 min.

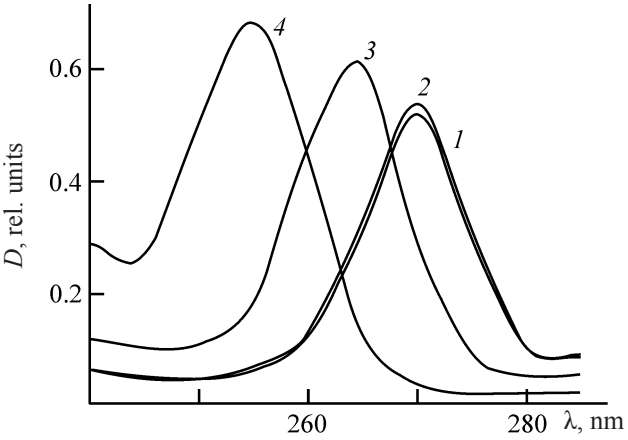

Fig. 1. UV spectra of acetic acid solutions of the compounds (*1*) CFZ + 10 AA, (*2*) CFZ + 70% AA, (*3*) CFZ + CTS + 10% AA, and (*4*) CFZ + CTS + 70% AA. Concentration 5×10^{-5} M. (*D*) Optical density and (λ) wavelength.

The antimicrobial activity was evaluated by the procedure described in [10]. The content of the antimicrobial substance in a standard disk was 30 mg. The admissible diameters (mm) of the growth suppression zones of control strains of microorganisms with a common nutritional demand were 29–37 mm.

The presence of a carboxy group in CFZ predetermines the possibility of its complexation with amino groups of CTS. The electronic spectrum of CFZ with a concentration of 10^{-4} M in a 1% aqueous solution of acetic acid contains a single absorption peak at 273 nm. Upon addition of chitosan to a solution of the antibiotic, the intensity of the absorption band of CFZ increases and its peak is shifted to shorter wavelengths by 5 nm. Chitosan does not absorb in this spectral range. The spectral changes indicate that chitosan affects the electronic system of CFZ and a complex is formed. In addition to complexation, such a shift in the electronic spectra can be caused by changes in the pH value of the solutions under study. However, varying the pH of the medium from pH 6 to pH 1 leads to only a slight decrease in the intensity of the absorption band of CFZ, whereas its position remains unchanged.

It was found using the method of isomolar series [11] that the ratio between the stoichiometric coefficients for the CTS : CFZ complex is 1 : 1. The formation of complexes is confirmed by ^{1}H NMR spectroscopic data. In the ^{1}H NMR spectrum recorded upon mixing of CTS and CFZ, the signals of protons at C^{22} and C^9 of the antibiotic are broadened by 1.0 and 0.2 ppm and downfield-shifted by 0.2 and 0.1 ppm, respectively.

Stability constants of the complexes and their content of complex-bound cefazolin at various acetic acid concentrations

Acetic acid concentration, %	$(\beta_c + 0.2) \times 10^{-5}$, M	Cefazolin content, wt %
0.1	3.8	17.0
1.0	4.5	15.9
10.0	6.3	4.5
70.0	10.1	3.7

In addition, the signals of protons at C^2 of chitosan are broadened by 0.02 ppm and downfield-shifted by 0.12 ppm. The strongest changes are observed for signals of protons situated in the immediate vicinity of the carboxy group of the antibiotic and the amino group of CTS. The spectral data obtained may indicate that the polysaccharide and the antibiotic interact by the scheme

$$\text{CFZ–COOH} + \text{H}_2\text{N–CTS}$$
$$\rightarrow \text{CFZ –COOH–H}_2\text{N– CTS}.$$

It is known [12] that an increase in the AA concentration changes the structure of CTS molecules in solution. This may affect the stability of the complexes being formed. The stability constants of the complexes in acetic acid with various concentrations were determined by the molar ratio method (MRM) [11] (see table). The data in the table demonstrate that an increase in the pH of the medium makes higher the stability of the complexes and their content of CFZ. To determine why this effect occurs, electronic spectra of CFZ were measured at different concentrations of AA and constant content of chitosan.

It can be seen in Fig. 1 that raising the AA concentration has nearly no effect on the position and intensity of the absorption peak of CZF, whereas addition of the polysaccharide to these solutions leads to an increase in the intensity of the absorption band of the antibiotic and to its hypsochromic shift by 4 to 10 nm. Apparently, this is primarily due to a variation of the supramolecular structure of the biopolymer solution with the AA concentration. CTS solutions in diluted AA (0.1 and 1%) are characterized by a compact form of CTS macromolecules. As the acid concentration is raised to above 10%, amino groups of the polymer are screened and its macromolecules are compacted. CTS solutions in 70% AA exhibit a high degree of solution structuring, which is

due, first, to the high degree of structuring of the solvent itself and, second, to the formation of a large number of intermolecular hydrogen bonds [13]. Thus, an increase in the acid concentration can change the CTS structure from dense, compact conformations of the macromolecule in the 1% solution to swollen macromolecular coils in 70% AA, and just this circumstance probably affects the stability of complexes formed by the biopolymer and antibiotic.

Complexes of CTS with CFZ were synthesized in the presence of acetic acid in various concentrations, isolated, purified, and characterized by means of elemental analysis and IR, UV, and 1H NMR spectroscopies. An increase in the concentration of acetic acid in solution is accompanied by a substantial decrease in the CFZ content of the complex, which can be understood with the degree of protonation of amino groups of chitosan taken into account (see table). The lower the degree of protonation, the higher the basicity of the amino groups and the greater the amount of the medicinal compound in the reaction product.

Data on the content of the antibiotic in the complex correlate with the results of an IR spectroscopic study. In the spectra of the complexes, the absorption band in the range 3000–3500 cm^{-1}, associated with stretching vibrations of OH and NH groups involved in hydrogen bonding, is broadened as compared with the corresponding bands of the antibiotic and chitosan. In the IR spectra of the polymeric complexes, there appear absorption bands at 1750 and 1710 cm^{-1}, associated with stretching vibrations of C=O and C=N groups of the medicinal compound. In addition, the absorption band of the C=O group of CFZ is shifted to shorter wavelengths (1720 cm^{-1}) as the AA concentration increases. The intensity of the absorption band of the C=O group of CFZ decreases on passing to complexes synthesized in the presence of higher concentrations of acetic acid, which is due to the stronger protonation of amino groups of chitosan in a more acidic medium and to the accordingly lower content of CFZ in the complex. Thus, chitosan-based complexes with a CFZ content of 3.7 to 17.0 wt % can be obtained by varying the reaction conditions.

When a medicinal preparation is incorporated into the polymeric matrix, its capacity to be retained within the film is determined by at least two factors. The first is directly the supramolecular structure of the polymer, determined by the process of film preparation. The second is the strength of the binding between the polymer and the medicinal substance. The release of the CFZ antibiotic from CTS-based films produced from solutions in acetic acid was studied at their different concentrations and a constant content of the antibiotic (1.3×10^3 M). Figure 2 shows typical kinetic curves of release of the medicinal compound from films formed in 1, 10, and 70% AA and treated with sodium dodecyl sulfate. It can be seen that CFZ is released most completely from the complex synthesized in the presence of 1% AA, whereas the release of the medicinal substance from the complex formed in 70% AA is slower and incomplete. Probably, this is due not only to formation of a water-insoluble layer constituted by the surfactant and the polymeric complex, but also to the different stabilities of complex compounds formed in deposition of a film, i.e., the higher the stability of a complex, the slower and less complete the release of CFZ. Apparently, the stability of the complex correlates with the kinetics of release of the medicinal preparation from films.

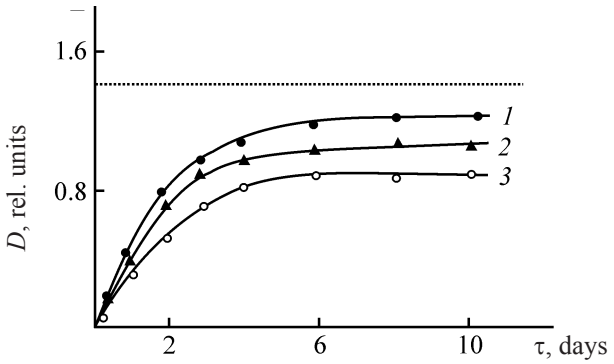

Fig. 2. Kinetic curves of cefazolin release from films of the complex CFZ + CTS, produced in the presence of (*1*) 1, (*2*) 10, and (*3*) 70% AA. CFZ concentration in the film 0.1 mol mol^{-1}; the optical density corresponding to this content of CFZ is shown by the dotted line. (*D*) Optical density and (τ) time.

The antimicrobial activity of the films obtained toward the most frequent burn wound infestant, Staphylococcus aureus (17 strains), was analyzed. The fraction of strains sensitive to the samples under study was determined to be about 70%.

Thus, the results of the study demonstrate that the interaction of CTS and CFZ mainly occurs at amino groups of the polysaccharide and carbonyl groups of the medicinal compound. The complexation process is strongly affected by the supramolecular structure and accessibility of reactions centers of the biopolymer. Variation of the conditions in which the films are formed and modified makes it possible to form polymeric film coatings with controlled transport properties and high antimicrobial activity.

CONCLUSIONS

Some fundamental aspects of the complexation of chitosan with cefazolin were determined and conditions of controllable release of the medicinal compound from the polymeric coating were found.

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