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Research paper

Polycomplexes of poly(acrylic acid) with streptomycin sulfate and their antibacterial activity

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Abstract

Complex formation between streptomycin sulfate and poly(acrylic acid) has been studied in aqueous solutions by turbidimetric, potentiometric and viscometric methods as well as by FTIR spectroscopy. It was shown that these polycomplexes are stabilized by electrostatic interactions. The solubility of polycomplexes was examined as a function of pH and it was found that at pH values below 3.1 the polycomplexes undergo complete dissociation or dissolution. The antimicrobial activity of the drug and its polycomplex was evaluated using *Sarcina sp.* as a model organism. It was demonstrated that the polycomplexes have an antimicrobial activity on the same level as the free drug.

Keywords: Streptomycin sulfate; Poly(acrylic acid); Polycomplexes; Antibiotic; Drug delivery

1. Introduction

Development of drug delivery systems (DDS) providing controlled and sustained release of a medicine to a specific site of the body has attracted considerable interest from pharmacists. When a dosage form is supposed for oral administration the drug formulation should be pH-sensitive since it passes through different sections of gastrointestinal tract with various pH values. A lot of different approaches have been tried in the design of different DDS such as the use of pH-sensitive hydrogels[1,2], beads[3], microspheres[4,5], polymeric films and enteric coating materials[6], etc.

Specific interactions of low molecular weight drugs with synthetic water-soluble polymers in aqueous solutions are of special interest for the development of drug delivery systems. These interactions lead to the formation of polycomplexes, which can be stabilized by ionic contacts, hydrogen bonding or hydrophobic forces[7–10]. The solubility of polycomplexes in most cases is limited to a certain pH range; therefore, this property can be utilized in the design of pH-sensitive dosage forms. The strong bonding of drug molecules to the macromolecules in these systems can also delay their release from the polymer matrix and provide a prolongation effect.

There have been many reports [11–13] of the development of DDS loaded with antibiotic medicines based on polymeric formulations consisting of poly(acrylic acid). They demonstrated that specific interactions between the polymer and a drug affect its release characteristics significantly.

Streptomycin is an antibiotic medicine, which is formed as a metabolic product of *Streptomyces globisporos streptomycini* or another similar organism[14]. It is active against a wide range of bacteria and is especially used in the treatment of tuberculosis. The major disadvantages of streptomycin are inadequate penetration into the cells due to its hydrophilicity, rapid elimination due to both efficient renal filtration and low level of association to plasma

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proteins, as well as severe adverse effects such as permanent deafness and renal toxicity[15].

Hofman and co-workers [16] reported the synthesis of a macromolecular streptomycin derivative in order to optimize its pharmacokinetics. They coupled streptomycin to methacrylic acid hydrozide and the resulting product was copolymerized with methacrylamide. This resulted in a non-degradable vinyl-type copolymer carrier. Schacht and co-workers [15] reported the synthesis of macromolecular streptomycin derivative by covalent coupling of the drug with polyglutamine and dextran. The hydrolytic stability of the polymer–streptomycin conjugate was studied at physiological and lysosomal pH (pH 7.4 and 5.2, respectively). Streptomycin release was found to be faster in the lysosomal pH range.

Several authors [17–19] reported on interactions of streptomycin with some acrylic acid derivatives including ion exchange resins. However, to the best of our knowledge there are no studies devoted to the complex formation of linear poly(acrylic acid) and streptomycin.

In the present work we studied the complex formation between linear poly(acrylic acid) (PAA) and streptomycin sulfate (SS) in aqueous solutions, evaluated the solubility of polycomplexes at different values and examined the antibacterial activity of the polycomplex.

2. Materials and methods

2.1. Materials

PAA (Product No 41,600-2) with an average molecular weight M_w of 2.5×10^5 Da was purchased from Aldrich Ltd.

SS was purchased from Khimpharm Ltd. (Shimkent, Kazakhstan) and used without further purification.

HCl and NaOH of analytical grade were obtained from Sigma and used as received.

2.2. Study of complexation

Titration was performed as a gradual addition of a solution of SS to a solution of PAA with constant stirring.

Viscosity of solutions was measured with an Ubbelohde viscosimeter at 25 ± 0.1 °C with a flow time of distilled water of 113 s.

Turbidity of solutions was measured with an UV2401PC spectrophotometer (Shimadzu, Japan) at a wavelength of 400 nm.

The pH of solutions was determined using an Ion Meter 3345 (Jenway Ltd., UK) with an accuracy of ±0.01 pH. In experiments on the effect of pH on the turbidity of polycomplexes the pH was adjusted by addition of insignificant amounts of 0.1 M HCl or NaOH to the solutions. The turbidity of solutions at different pH values was determined using an Ion Meter 3345 (Jenway Ltd., UK) and UV2401PC spectrophotometer (Shimadzu, Japan).

2.3. Spectroscopic characterization

FTIR spectra of starting components and polycomplexes were recorded with a FTIR Satellite Spectrophotometer (Matson, USA) in KBr tablets. For spectral investigations the precipitate of polycomplex obtained by mixing aqueous solutions of PAA and SS was centrifuged, washed by small amounts of distilled water and dried in a vacuum.

2.4. Microbiological testing

Sarcina spp. germs culture was obtained from the Museum of culture collection, Laboratory of Microbial Pathology (All-Union Collection of Microorganisms, Moscow, Russia). It was used for evaluation of antibacterial activity of SS and its polycomplex.

Microbiological evaluation of antibacterial activity of SS and the polycomplex was performed by the limiting dilution method. Beef-extract powder purchased from DIFCO (USA) was used as a liquid nutrient medium. For preparation of the liquid nutrient medium 40 g of beef-extract powder was mixed with 1000 ml of distilled water and then the mixture was boiled with stirring until complete dissolution. Then the nutrient medium was cooled, poured into test tubes, which were closed by cotton wool stoppers and covered by dark paper. The test tubes were autoclaved to ensure the medium was sterile. The bacterial growth is inhibited outside a pH range of 5.6–7.0, thus the pH of nutrient medium was adjusted to 6.0.

The incubation was conducted at 35–37 °C for 24 h with thermostatic control and registered visually through the appearance of turbidity.

3. Results and discussion

3.1. Formation of polycomplexes

Streptomycin sulfate is a triacidic base and its structure allows the possibility of non-covalent interaction of the drug with anionic polymers through ionic contacts.

Poly(acrylic acid) is one of many water-soluble polymers used for the development of different pharmaceutical formulations[11–13,20,21]. It is a weak anionic polyelectrolyte with the degree of dissociation strongly dependent on pH. The derivatives of PAA are widely used as suspending agents in pharmaceutical preparations, binding agents in tablets and as bioadhesive matrixes, etc.

Mixing of aqueous solutions of PAA with SS is accompanied by the appearance of turbidity (D). Fig. 1 shows turbidimetric titration curves of PAA by solutions of SS with different concentrations of both components. The appearance of turbidity is caused by the formation of a low

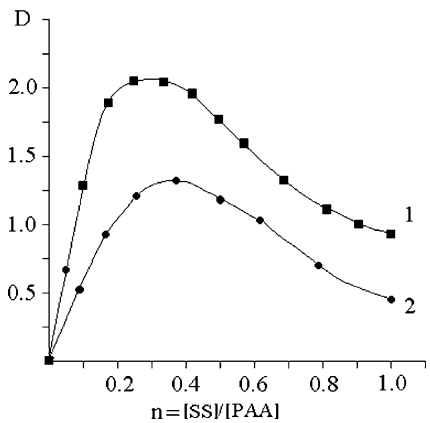

Fig. 1. Turbidimetric titration curves of PAA solution by SS solution. $C_{SS} = C_{PAA} = 0.1$ (1), 0.05 g/100 ml (2).

solubility product of hydrophobic nature with further aggregation of polycomplex particles. The point of maximum turbidity corresponds to the ratio in which the components form the polycomplex and does not depend on the concentration of the starting components.

The results of potentiometric titration of PAA by solutions of SS are presented in Fig. 2. At the beginning of the titration a considerable decrease in pH was observed. This was caused by ion exchange as a result of complex formation. After the required ratio of the components in the polycomplex was achieved, the pH increased slightly because the solution was more diluted due to the presence of excess of SS solution in the mixture.

When the concentration of the components was 0.1 g/100 ml the mixture was turbid without precipitation of the polycomplex. This allowed us to measure their viscosity. The addition of SS to PAA leads to a drastic decrease in solution viscosity, indicating that the polycomplex of PAA-SS has a relatively compact structure with particle sizes that are smaller than the hydrodynamic volume of PAA coils (Fig. 3). In a blank experiment the solution of PAA was diluted by pure water. In this case, an

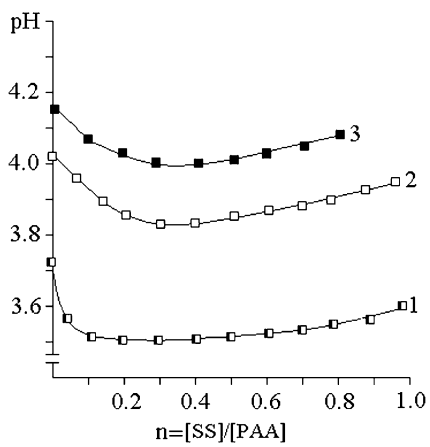

Fig. 2. Potentiometric titration curves of PAA solution by SS solution. $C_{SS} = C_{PAA} = 0.1$ (1), 0.05 (2), 0.025 g/100 ml (3).

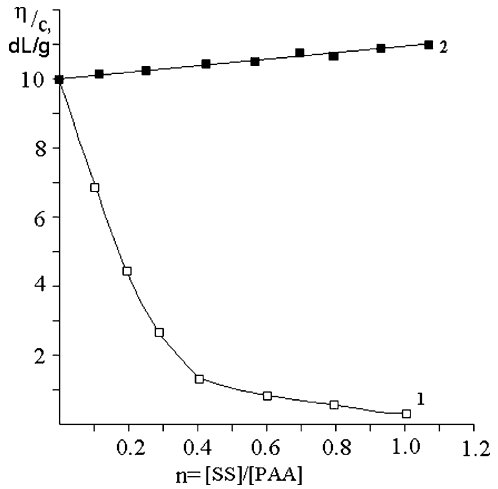

Fig. 3. Viscometric titration curves of PAA solution by SS solution (1) and water (2). $C_{SS} = C_{PAA} = 0.1$ g/100 ml.

increase in reduced viscosity was observed. This was caused by the unfolding of the macromolecules due to the polyelectrolyte effect.

In summary, the results of the turbidimetric, potentiometric and viscometric titrations are in clear agreement and indicate the formation of polycomplexes with composition [SS]:[PAA] = 0.3–0.4 g/g, i.e. 0.3–0.4 g of antibiotic is bound by 1 g of PAA. It should be noted that the composition of the polycomplex is not changed upon variation of SS and PAA concentration within 0.025–0.100 g/100 ml.

The solubility of PAA–SS polycomplexes was evaluated as a function of pH by a turbidimetric method. Fig. 4 shows the dependence of PAA–SS mixture turbidity on pH. It is seen that an increase in pH leads to a sharp rise of turbidity in the pH range 3.15–4.2. This observed trend is probably caused by an increase in PAA dissociation at the higher pH region and enhancement of the ability of macromolecules to bind the drug. At pH values <3.1 the solution mixture is clear indicating the complete destruction of the polycomplex to the starting components or its solubilization in water. The low solubility of polycomplex at the higher pH region gives a hypothesis that upon

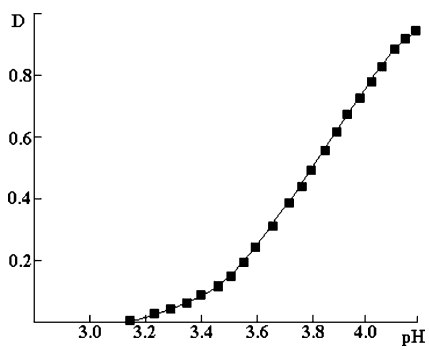

Fig. 4. Dependence of polycomplex SS–PAA (2:1 g/g) solution turbidity on pH. $C_{SS} = C_{PAA} = 0.1$ g/100 ml.

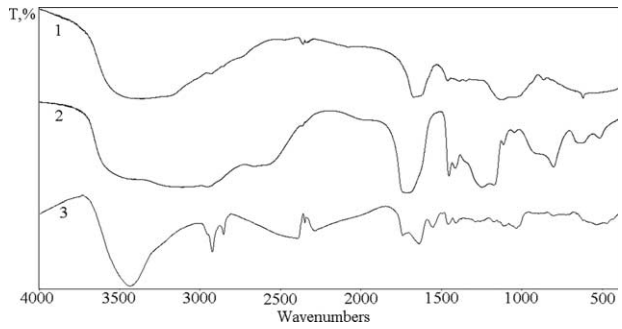

Fig. 5. FTIR spectra of SS (1), PAA (2) and polycomplex (3).

administering this polymeric form into the intestine it will release the free drug very slowly providing a sustained release profile. However, during passage through the stomach the polycomplex may dissociate and release the drug before reaches the intestine. Therefore, the development of an acidic insoluble coating is required for protection of the polycomplex. The research work on the development of the dosage forms based on SS–PAA polycomplexes is in progress and will be reported in further publications.

In order to perform spectral characterization of the polycomplex it was separated from solution, washed by small volumes of water and then dried under vacuum. The FTIR spectra of PAA, SS and the polycomplex are presented in Fig. 5. The bands at 3204 and 3375 cm^{-1} are typical for hydroxyl- and primary amino-groups and at 1061 cm^{-1} the ether bonds are clearly seen in the spectrum of SS. The bands at 3170 and 1707 cm^{-1} seen in the spectrum of PAA are typical for hydroxyl and carboxyl groups, respectively. The spectrum of the polycomplex is characterized by the presence of the bands typical for both components confirming their co-precipitation within one compound. Apart from the appearance of the new band at 1558 cm^{-1}, which shows the presence of carboxylate anion in the polycomplex, a shifting of the band at 1413 cm^{-1}, which was typical for PAA to the lower region (1406 cm^{-1}) also confirms the ionization of PAA in the course of its complexation with SS. Thus, based on the results obtained we can conclude that the polycomplexes formed between SS and PAA are mainly stabilized by electrostatic forces.

3.2. Microbiological testing of polycomplexes

In order to evaluate antimicrobial activity of the polycomplexes (PC) and starting components we have performed the microbiological tests using *Sarcina sp.* as a model germ culture. The growth of the germ culture was studied in the presence of SS and PC of different concentrations. According to the results obtained (Table 1) the minimal inhibition concentration of the drug with respect to *Sarcina sp.* is observed at 3.12×10^{-6} g/l of SS. The same value was found for polycomplex of streptomycin with PAA. It should be noted that in a control experiment with PAA alone the bacterial growth was not inhibited.

The effect of the antibiotic and its polycomplex on germs culture of *Sarcina sp.* is shown in Fig. 6. In test tube 1 containing pure SS the nutrient medium is clear, which confirms the inhibition of the bacterial growth. Test tube 2 is turbid because of intensive bacterial growth in the absence of the drug. Test tube 3 contained the polycomplex in the form of dispersion, the growth of bacteria was also inhibited. The polycomplex remained insoluble because the pH of the nutrient medium was fixed at 6.0.

Hence, the polycomplex of SS with PAA has a level of activity that is very close to the free drug. As shown in the previous section the polycomplexes of SS with PAA are mainly stabilized by electrostatic interactions and their formation/dissociation can be described by the following equilibrium:

$$SS + PAA \Leftrightarrow PC$$

This equilibrium is mobile because electrostatic forces are very sensitive to environmental parameters such as component concentration, pH and ionic strength of solution. A slight variation in environmental parameters can shift the equilibrium causing the dissociation of the polycomplexes and wash out of the free drug. It is likely that in aqueous media the polycomplex is partially

Table 1
Inhibition of germ culture *Sarcina sp.*by streptomycin sulfate and its polycomplex with poly(acrylic acid)

No.	1	2	3	4	5	6	7	8	9	10	11	12	13[a]	14[b]
?$_{SS,}$ (mkg/ml)	200	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.1	0	0
Germs culture growth														
SS	−	−	−	−	−	−	−	+	+	+	+	+	+	−
PC	−	−	−	−	−	−	−	+	+	+	+	+	+	−

+, Presence of germ culture growth; −, absence of germ culture growth.
[a] Control of germ culture growth.
[b] Control of sterility of nutrient medium.

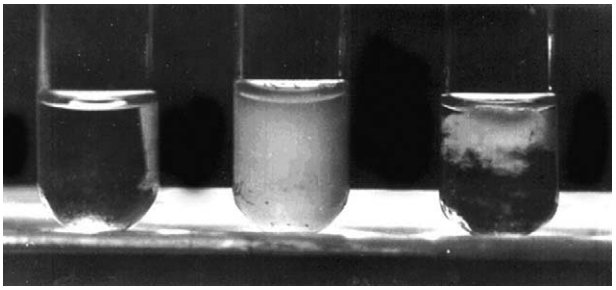

Fig. 6. Effect of SS (1) and SS–PAA polycomplex (3) on germs culture *Sarcina spp*. Test tube 2 is a blank experiment.

dissociated with release of free drug molecules, which have the same antimicrobial activity as the drug without PAA.

4. Conclusion

Interaction between streptomycin sulfate and polyacrylic acid in aqueous solutions led to the formation of low-soluble polycomplexes stabilized by electrostatic forces. The structure of the polycomplexes is compact and their stability/solubility is greatly dependent on the pH. The polycomplexes are shown to be destroyed at a pH lower than 3.1. In experiments with the inhibition of model germ culture growth, it was shown that the polycomplexes have the same level of antimicrobial activity as the free drug.

The information obtained in the present work can be used for the design of new drug delivery systems and can be also taken into account for the prediction of drug interactions within pharmaceutical formulations.

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