Cefalosporin complexes with the methylene blue dye and their use in the surgery of infectious endocarditis

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Complexation of antibiotics cefazolin and cefoperazone with the methylene blue thiazine dye was studied. Composition (1 : 1), stability constants (150±30 M^{-1}), and solubility of the complexes were determined.

Key words: cefalosporins, cefazolin, cefoperazone, methylene blue, complexes, infectious endocarditis.

Infectious endocarditis belong to the severe and widespread heart diseases. In the last 20 years, morbidity of the infectious endocarditis increased almost three-fold and amounts from 3.1 to 16 cases per 100.000 of population.[1—3] Despite increasing arsenal of efficient antibiotics with wide range of action, the problem of successful interruption of the infection process by antibacterial therapy is not still solved. Lethality from infectious endocarditis during surgical treatment (in combination with antibacterial therapy) remains high: from 11 to 40%. The most frequent reason for unsatisfactory results of the surgery is an infection backset in the form of prosthetic endocarditis, which is observed in 3.1—18% of cases, that is by 3—5 times higher than the frequency of such complications after surgeries on substitution of a cardiac valve for the heart diseases of other etiologies. The after-surgery mortality rates for surgical procedures on prosthetic endocarditis reach 20—60%. The nidus of microbial invasion of infectious endocarditis is a native heart valve, for prosthetic endocarditis, the valve prosthesis, therefore, in the last years significant attention is paid to the local use of antibacterial medicines and antiseptics. In this case, the main problem consists in the prolongation of their action, which can be solved by the creation of a depot of medications (through the decrease of their solubility) directly in the endocardium tissues and the cuff of the heart valve prosthesis.

The main medicines for treatment of endocarditis are antibiotics of cefalosporin series, *viz.*, cefazolin (**1**) and cefoperazone (**2**).[4] At the same time, the methylene blue thiazine dye (MB, **3**) possesses antiseptic properties, is permitted for the intravenous introduction,[4] and forms complexes with the salts of organic acids.[5,6] Since cefazolin and cefoperazone are sodium salts of organic acids, the purpose of the present work consisted in the study of complexation of cefazolin and cefoperazone with the MB dye, determination of composition and stability constants of the complexes, as well as solubility under physiological conditions.

Experimental

Spectra in the UV and visible regions were recorded on a Specord M-40 spectrophotometer. Cefazolin and cefoperazone (sodium salts) of pharmacopoeia purity were used in the work, methylene blue was purchased from Merk.

Stability constants (K_{stab}) of complexes and stoichiometric coefficients of the complexation reactions were determined according to the procedures given in the works.[6,7]

Complex cefazolin—methylene blue (4). A solution of hydrate **3**·3H_2O (0.747 g, 2.0 mmol) in H_2O (40 mL) was added to cefazolin (1 g, 2.1 mmol) in H_2O (20 mL) with stirring. A precipitate was separated by centrifugation, washed with water several times at 0 °C. The precipitate was dried first in air, then at 105 °C until the weight was constant to obtain complex **4** (1.4 g, 95%) as bright violet crystals poorly soluble in H_2O and EtOH. Found (%): C, 46.98; H, 4.68; N, 19.99; O, 11.05 (calculated on the residue); S, 17.30. Calculated (%): C, 48.84; H, 4.21; N, 20.90; O, 8.68; S, 17.37.

Complex cefoperazone—methylene blue (5). A solution of hydrate **3**·3H_2O (0.523 g, 1.4 mmol) in H_2O (30 mL) was added to cefoperazone (1 g, 1.5 mmol) in H_2O (15 mL) with stirring. Further treatment as for complex **4** yielded complex **5** (1.20 g, 92%) as violet plates poorly soluble in H_2O and EtOH. Found (%): C, 54.00; H, 5.21; N, 15.90; O, 14.88 (calculated on the residue); S, 10.01. Calculated (%): C, 54.37; H, 4.85; N, 16.61; O, 13.81; S, 10.36.

Determination of the complexes 4 and 5 composition. An exactly weighed sample (5—10 mg) of the corresponding complex

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was dissolved in the known volume of H_2O (5—10 mL). The content of **3** was determined according to the State Pharmacopy XI: an excess of aqueous potassium dichromate was added to a solution of the complex, a precipitate that formed was centrifuged, the unreacted potassium dichromate was determined iodometrically. The content of cefalosporin was determined from the difference between the sample weight and the content of dye **3**.

Determination of the complex solubilities. An aliquot was taken from the prepared saturated solution of the complexes in 0.15 *M* NaCl at 37 °C, the content of the dye **3** in the aliquot was determined using potassium dichromate.

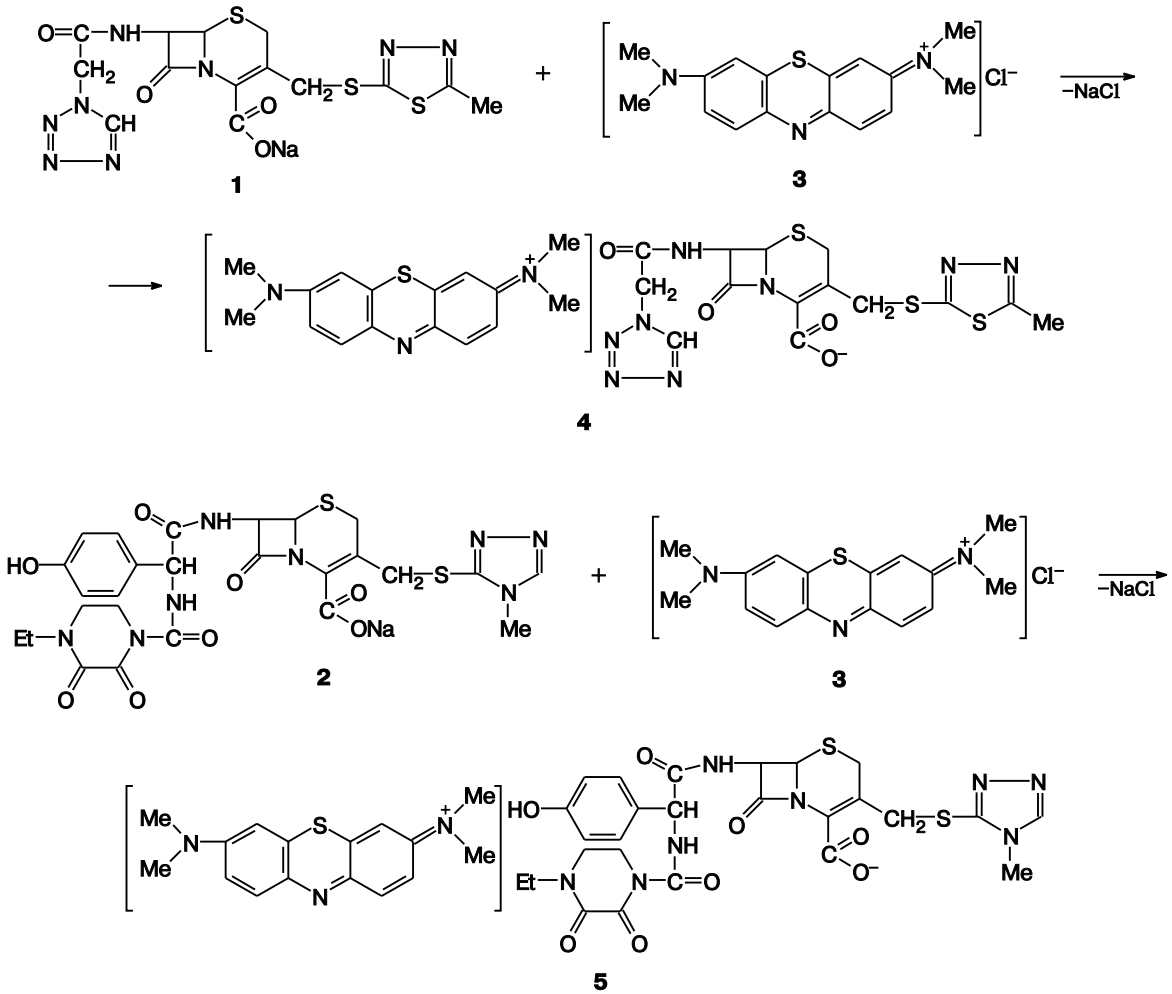
Results and Discussion

Electron absorption spectrum of MB with its concentration in water of 10^{-4} mol L^{-1} is characterized by the presence of two absorption maxima at 600 and 665 nm (Fig. 1, curve *1*). The first maximum belongs to the MB molecules in the dimeric form, the second — to the MB molecules in the monomeric form.[8]. When excess of other component, cefazolin (or cefoperazone), is added to the solution of MB, the absorption peak of the dimer considerably decreases, the absorption maximum of the monomer initially increases (Fig. 1, curve *2*) and then, with the increase of the antibiotic concentration, also decreases together with appearance of a new absorption band at 675—680 nm (Fig. 1, curves *3* and *4*). The spectral changes indicate a strong effect of the cefazolin anion on the π-electron system of MB and the formation of the charge-transfer complex (Scheme 1). As it is seen from Fig. 1, the extinction coefficient of complex **4** is lower than that of the dye.

When the concentration of MB is 10^{-5}—10^{-6} mol L^{-1}, there is virtually no a dimer form of the dye in the solution,[6] and in this case it becomes possible to determine K_{stab} and stoichiometric coefficient of the complexation reaction by the method of molar proportions.[7]

If the concentration of MB is constant ($4.74 \cdot 10^{-5}$ mol L^{-1}, for this concentration $A_{665} = 0.39$), as well as if we neglect existence of the dimer in very dilute solutions and under conditions of a large excess of the

Scheme 1



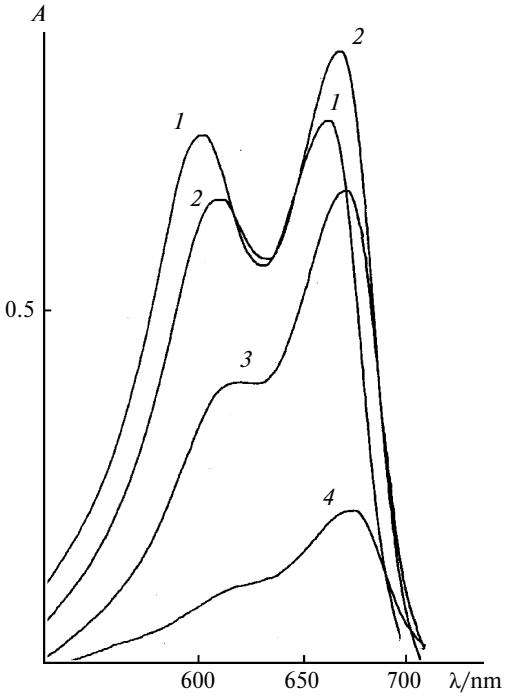


Fig. 1. UV-visible spectra of the aqueous solutions of MB (*1*) ([MC] = 10^{-4} mol L^{-1}) in the presence of growing concentrations of cefazolin (**1**): $5 \cdot 10^{-4}$ (*2*), 10^{-3} (*3*), $4 \cdot 10^{-3}$ (*4*).

changeable concentration of the second component R ($5.30 \cdot 10^{-4}$—$5.30 \cdot 10^{-3}$ mol L^{-1}), the K_{stab} for both complexes was found to be equal to 150±30 M^{-1} from the graph of the dependence of $[MB]_0/A - A_0$ from $1/[R]$[7,8] (A and A_0 are the absorption of solutions in the presence and absence of component R, respectively, $[MC]_0$ is the initial concentration of MB, [R] is the concentration of cefazolin or cefoperazone). The stoichiometric coefficient[7] of the complexation reaction was ~0.8.

When concentration of MB > 10^{-3} mol L^{-1}, addition of an aqueous solution of the corresponding cefalosporin to the solution of MB led to the formation of complexes (see Scheme 1) as bright violet precipitates. Results of elemental analysis and determination of MB by the dichromate method showed that each complex contained MB and cefalosporin in equimolar amounts.

Solubilities of complexes **4** and **5** under conditions close to physiological were $8.5 \cdot 10^{-4}$ mol L^{-1} and $1.9 \cdot 10^{-4}$ mol L^{-1}, respectively. With allowance for the 1 : 1 composition of the complexes, the corresponding solubility products were $7.2 \cdot 10^{-7}$ and $3.6 \cdot 10^{-8}$ mol^2 L^{-2}.

Both complexes showed ability to form associates in aqueous medium: the electron absorption spectra in the visible region, together with the absorption maximum of the monomeric form at 665 nm, exhibited the second maximum at 600 nm characteristic of the dimeric form ([complex] > 10^{-5} mol L^{-1}). From this it follows that the complexes, like MB itself, possess properties of dyes. The spectra of the complexes showed changes in the UV region, but these changes were additive with respect to the starting MB and the corresponding cefalosporin. This indicates that the structures of cefazolin and cefoperazone did not change. The testing showed that the complexes exhibited the same antimicrobal activity as cefalosporins themselves.[9]

To sum up, physicochemical characteristics of complexes **4** and **5** allow one to use them as dyes and prolonged forms of antibiotics.

To treat the endocardium and prosthesis heart valve tissues, the complexation of cefazolin with MB was used *in situ*. First, the tissue was colored with 1% solution of MB, then a solution of antibiotic (0.25 g mL^{-1}) was deposited, and then again MB to reach a high concentration of the complex on the surface of endocardium or tissue of the heart valve prosthesis. The procedure was introduced into the surgery clinical practice for patients with the active form of infectious endocarditis and for prevention of prosthetic endocarditis for routine patients with an acquired heart valvular disease (Republican Cardiology Clinic, Ufa). From 2002 until the present time, 87 patients with infectious endocarditis (aged from 10 to 64 years) were satisfactory cured without backsets after surgeries with the use of our procedure, no cases of prosthetic endocarditis (from more than 200 surgical patients) were observed.

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