SPECTRAL STUDY OF MITOXANTRONE WATER-SALINE SOLUTIONS

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Self-association process of mitoxantrone molecules in water-saline solution depending on concentration, temperature and solution ionic strength has been studied. It was shown that in water-saline solutions associates of mitoxantrone molecules are formed and their domination (stacking-like or molecules are bound by hydrogen bonds) strongly depends on not only mitoxantrone concentration, but also temperature and solution ionic strength.

Keywords: mitoxantrone, self-association, water-saline solution, absorption spectrum.

Introduction. Mitoxantrone (see Scheme) is an anticancer drug that has been successfully used in cancer chemotherapy [1–3]. Like anthracyclines (dynamitsin, adriamycin etc.) mitoxantrone (MTX) binding with DNA changes chromatin structure [4] and DNA compaction [5], moreover it possesses relatively low toxicity compared with anthracyclines, by which preferable application of MTX in clinics is conditioned [3, 6]. The interaction of MTX with double-stranded nucleic acids is spectrophotometrically studied [6–16]; DNA–MTX binding thermodynamic parameters are determined [6, 9, 15, 16]. In [5, 6, 8, 13, 15, 16] the quantitative investigation of MTX–DNA complexes is carried out at relatively high concentrations of MTX, where the formation of self-associates of MTX molecules prevails [9, 17–19]. The modes of self-association of MTX molecules in water-saline solutions were studied in details by NMR method. It was shown that in the process of self-association stack-shaped structures stabilized by associates (mostly dimers) were bound by intermolecular hydrogen bonds and interaction of aromatic rings were formed [19]. In these studies [9, 17–19] the effect of ionic strength and temperature on MTX self-association character was not studied and MTX maximal concentration, when the formation of dimers can be neglected, was not revealed. It should be noted that the formation of the above-mentioned various types of associates changes the absorption spectrum of MTX [17].

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In order to reveal how the self-associates influence on DNA–MTX complex formation, the association process of MTX molecules in water-saline solution depending on MTX concentration, temperature and ionic strength has been studied using UV-VIS spectroscopic method.

**Materials and Methods.** Mitoxantrone (“Sigma”, USA), NaCl, Tris (“Serva”, Germany) and double-distilled water were used in experiments. MTX concentration was determined spectrophotometrically, using the value of monomeric state extinction coefficient $\varepsilon_{659}=25090 \text{ M}^{-1}\text{cm}^{-1}$ [18]. All preparations were used without further purification. Electronic absorption spectra of MTX were recorded in 400–800 nm range on the spectrophotometers Unicam SP8-100 (England) and Cary-219 (USA). Thermostating was realized using a thermostat Haake-F3 (Italy). Studies were carried out in an aqueous solution containing $0.1 \text{ M NaCl} (\mu)$, $0.01 \text{ M Tris}$ and $5 \times 10^{-4} \text{ M EDTA}$ (pH 7.4). Measurements were carried out in temperature-controlled cells using quartz cuvettes with 10 mm volume and tight-fitting stoppers. The accuracy of determining the temperature was $\pm 0.05 ^\circ\text{C}$ and optical density was $10^{-4}$ optical units.

**Results and Discussion.** We consider the association process of MTX molecules with increasing of its concentration at the constant temperature $(34 ^\circ\text{C})$ and $\mu=0.11 \text{ M NaCl}$. Fig. 1 shows the variation of the absorption spectra at increasing of MTX concentration in the solution. As it follows from Fig. 1, with increasing of MTX concentration, the absorption maxima at 665 and 611 nm are not shifted the optical density of the solution increases only. Moreover, with increasing concentrations of MTX, a relative increase in absorbance at 665 nm is much smaller than 611 nm. Consequently, the absorbance at 665 nm is likely to characterize the monomeric state of the molecules MTX, and the maximum absorbance at 611 nm of associated state. In Fig. 1 the dependence of the optical density at 659 nm ($A_{659}$) on total MTX concentration ($C_0$) in solution is presented. As it follows from Fig. 2, with $C_0$ enhancement, $A_{659}$ increases approximately linearly in $0<C_0 \leq 3 \times 10^{-6} \text{ M}$ interval, and then disrupts linearity that can indirectly indicate the formation of associates. Therefore, $\mu=0.11 \text{ M NaCl}$ and temperature of $34 ^\circ\text{C}$ at concentrations greater then $3 \times 10^{-6} \text{ M}$, association process becomes noticeable and they cannot be neglected.
The phenomenon of spontaneous association of MTX molecules in solution is clearly detected from the analysis of the temperature dependence of the absorption spectra in the visible region of the spectrum (Fig. 3). As it follows from Fig. 3, at a constant concentration of MTX ($C_0=6.2 \times 10^{-6} M$) and $\mu=0.11 \ M \ NaCl$, with an increase in temperature the optical density of the solution increases, wherein the wavelength of maximum absorption is not changed. Simultaneously isosbestic point is observed at 695 nm, which is not altered with increasing temperature. The presence of isosbestic point or points indicates that there is a superposition of two independent absorption spectra.

Fig. 3. MTX absorption spectra ($C_0=6.2 \times 10^{-6} M$) at different temperatures at $\mu=0.11 \ M \ NaCl$: 1 – 28.5°C; 2 – 48.5°C; 3 – 63.2°C.

At MTX tested concentrations ($C_0=6.2 \times 10^{-6} M$) in solution there are mainly monomers and dimers. As the temperature increases, the fraction of molecules of MTX-dimers decreases. In [17, 19] it was shown that at the formation of stack-shaped associates, the maximum absorption wavelength was not changed, only the absorption decreased. With high probability, at studied ionic strengths MTX molecules form stack-shaped associates. The presence of an isosbestic point in the absorption spectra (Fig. 3) also indicates two states in the solution. From the absorption spectra shown in Fig. 3 follows that by temperature increasing from 28.5 to 63.2°C a relative absorption increase in the absorption maxima grows differently: $A_{665}$ approximately 1.8 times and $A_{661}$ increases 1.3 times. As the temperature increases, at a constant concentration $C_0$ of MTX in the solution the proportion of the monomers increases, it is possible to argue that the absorbance at 665 nm characterizes the state of the monomeric molecules of MTX.

Therefore, MTX molecules in the solution are located in two spectrophotometrically distinct states: monomeric and stack-shaped associated (quite possibly dimerized), the share of the latter decreases with increasing of the temperature in the studied temperature range (from 28.5 to 63.2°C).

The association process was also studied, depending on the ionic strength at a constant temperature (34°C). At constant $C_0=3.5 \times 10^{-6} M$ with increasing ionic strength from $3 \times 10^{-3}$ to $5 \times 10^{-3} \ M \ NaCl$ absorption of MTX solution decreases, and then with further increasing NaCl concentrations (0.25 $M$ to) absorbance...
monotonically increases (Fig. 4). Moreover, when the optical density decreases a red shift is observed on the absorption spectra in the visible region of the spectrum (≈ in 3 nm); \( \lambda_{\text{max}} \) is shifted from the value of 662 and 610 nm to 665 and 613 nm respectively. When the optical density begins increasing, the absorption maximum at 665 and 613 nm remains unchanged. In [9] the absorption spectra of MTX with increasing polarity of the solvent were investigated. It was shown that a decrease in solvent polarity \( \lambda_{\text{max}} \) (\( \lambda_{\text{max}} = 660 \) nm for MTX in double-distilled water) is shifted to longer wavelengths due to a decrease in the number of molecules of hydrogen bonds with water molecules and MTX. It should be noted that the red shift is observed upon binding MTX to DNA [6, 9, 15, 18]. Studies have also shown [17] that in aqueous solutions of phenolic OH groups, MTX forms hydrogen bonds with the molecules of polar solvent. At the same time by means of intermolecular hydrogen bonds can form molecular associates MTX [17, 19]. This ionic strength sets the balance between these kinds of associates which depend on the polarity of environment.

Taking into account the results of [17, 19] it can be assumed that an increase in the ionic strength from \( 3 \times 10^{-2} \) to \( 5 \times 10^{-3} \) M NaCl form two types of associates (stack-shaped and associated with intermolecular hydrogen bonds), which leads to a decrease in the optical density of the solution and red shift. A further increase in ionic strength likely reduces the proportion of stack-shaped associates with almost constant concentration of MTX molecules linked by intermolecular hydrogen bonds (in the field of NaCl concentration the red shift of the characteristic changes in the number of hydrogen bonds is observed).

Therefore, by increasing the ionic strength from \( 3 \times 10^{-2} \) to \( 5 \times 10^{-3} \) M NaCl a structure formed by associates of MTX molecules should differ from associates formed at higher ionic strengths. Summarizing the dependence of the absorption spectra of the concentration of MTX on the ionic strength and temperature of the solution, it can be assumed that in an almost physiologic conditions, increasing the concentration of MTX and reducing the solution temperature_MTX lead to the formation of stack-shaped associates (more likely dimers) and due to an increase of ionic strength changing water structure (polarity protection), which leads most likely to the formation also of longitudinal associates: MTX molecule by means of intermolecular hydrogen bonds forms associates.

**Conclusion.** From the comparison of experimental data obtained from the spontaneous association of molecules of mitoxantrone, it can be concluded that the aqueous salt solutions, where the interaction of mitoxantrone with nucleic acids is usually studied, almost always associates of molecules of mitoxantrone are formed, the structure of which (stack-shaped or molecules are connected by hydrogen bonds) strongly depends not only on the concentration of mitoxantrone, but also on the temperature and ionic strength. Moreover, if the conditions are close to physiological concentrations of mitoxantrone in the order of \( 3 \times 10^{-6} \) M, almost all molecules of mitoxantrone are in monomeric state, at lower ionic strengths they are at such low concentrations that can form associates. Moreover, the reduction in the ionic strength to 0.03 M NaCl concentration leads to increasing shaped stack associates, and further decreasing to 0.005 M NaCl, leading to a decrease in the shape of associates of both types (shaped stack and linked by hydrog.
REFERENCES


