Spectrophotometric investigation of the hetero-association of Caffeine and thiazine dye in aqueous solution

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Abstract

The self-association of thiazine dye, Methylene Blue (MB), and its hetero-association with Caffeine (CAF), were studied in aqueous solution by means of spectrophotometry in the visible range of spectrum. Concentration and temperature dependences of molar absorption of the interacting molecules were used to analyse dynamic equilibrium in solution in terms of two-component model of molecular hetero-association. The magnitudes of equilibrium dimerization and hetero-association constants as well as thermodynamic parameters, enthalpy and entropy, were determined. The calculation of the fraction of different types of associates in the mixed solution, containing Methylene Blue and Caffeine, was done. It was concluded that the hetero-association of Methylene Blue and Caffeine molecules results in lower effective concentration of the dye in solution, which may account for the alteration of its biological activity.
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1. Introduction

It is generally considered now that the hetero-association between biologically active aromatic molecules is an important process, which in certain cases may govern a biological activity of aromatic drugs given in combination [1–5]. The mechanism of changing of the biological activity due to the hetero-association is two-fold. First, a direct complexation between aromatic molecules may modify their pharmacokinetic properties or lead to a chemical degradation [1–3]. Second, the hetero-association between aromatic DNA intercalators decreases their monomeric concentrations in solution, thus making the fraction of DNA-bound intercalator lower which results in alteration of the observed biological effect [5–8].

Numerous studies carried out on cellular systems report a marked change of the biological activity of aromatic antibiotics and dyes in the presence of Caffeine (CAF, Fig. 1a) [4,5,9]. Investigations of the underlying mechanisms have proved the formation of stable hetero-complexes between the interacting molecules arranged in a sandwich-type manner in solution, which enabled to correlate the hetero-association and the alteration of the biological activity due to Caffeine [7,8,10,11]. It is, therefore, considered now that CAF provides a potential possibility to regulate the biological activity of aromatic dyes and antibiotics in vivo which is of particular importance for clinical applications [8,10].

Aromatic dye, Methylene Blue (MB, Fig. 1b), is a phenothiazine derivative, capable to bind with nucleic acids and, in particular, with virus nucleic acids [12]. Sensitivity of MB to different conformational transitions of DNA in cell nuclei allows to use it as a label for investigation of structural changes in biopolymers.

Methylene Blue is an active photosensibilizator and is utilized during photodynamic therapy of malignant tumors [13]. If Caffeine molecule is capable to intercept aromatic dyes via the hetero-association [7,8,10], it therefore, may be assumed that CAF can form non-covalent complexes with MB, thus altering the biological activity of the dye. The effect may be significant when consuming caffeinated products during the therapy.

In the present work, the ability of CAF to form non-covalent complexes with thiazine dye, Methylene Blue, was studied by means of UV–vis spectrophotometry.

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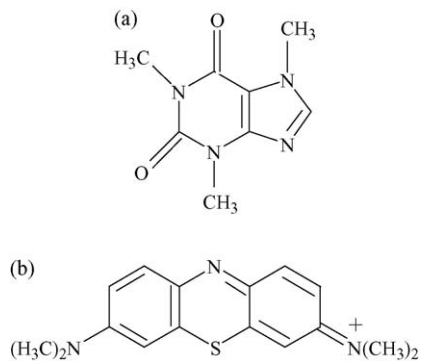*E-mail address:* max_evstigneev@mail.ru (M.P. Evstigneev).

Fig. 1. Structural formulas: (a) Caffeine and (b) Methylene Blue.

2. Materials and methods

Electronic absorption spectra of the investigated solutions of Methylene Blue and Caffeine ('Sigma') were measured using SF-46 spectrophotometer (LOMO, St. Petersburg, Russia) in the visible spectrum range (500–730 nm) at $T = 293$ K.

Solutions of MB for the self-association studies were prepared by sequential dilutions in 0.1 M phosphate buffer (pH 6.86) using bidistilled water in the range of concentrations 10^{-3} to 10^{-6} M. The measurements were made in standard glass cells with an optical path 2.0, 1.0, 0.2 and 0.01 cm.

During the hetero-association studies, the concentration of Methylene Blue in the mixed solution with Caffeine was maintained constant ($C_{MB} = 2.5 \times 10^{-4}$ M = constant). The range of CAF concentrations (1×10^{-4} to 5×10^{-2} M) were used for MB titration by the solution of Caffeine. The measurements were made in standard glass cells with an optical path 0.0207 and 0.0505 cm.

Thermodynamic investigations of the self- and hetero-association of MB and CAF were made in 1.0 cm cells in the temperature range 293–353 K. The temperature was stabilized in spectrophotometer cell compartment with an accuracy $\pm 0.15\,^\circ$C.

3. Results and discussion

3.1. Self-association of Methylene Blue in aqueous solution

Quantitative analysis of MB self-association was carried out using concentration dependence of the dye molar extinction coefficient on the wavelength of the absorption maximum of the molecules in monomer form ($\lambda_{max} = 664$ nm) (Fig. 2).

The self-association of the molecules was analysed in the region of low concentration of MB ($[D_0] < 2 \times 10^{-4}$ M) with an aid of a dimer model. The following scheme of molecular equilibrium in solution was considered:

$$D_1 + D_1 \overset{K_d}{\longleftrightarrow} D_2 \tag{1}$$
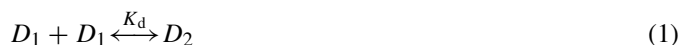
where K_d is an equilibrium constant of molecular dimerization; D_1 and D_2 are monomer and dimer forms of the dye, respectively.

The experimental concentration dependence of the dye molar absorption, ΔA_M, was analysed using the following equation
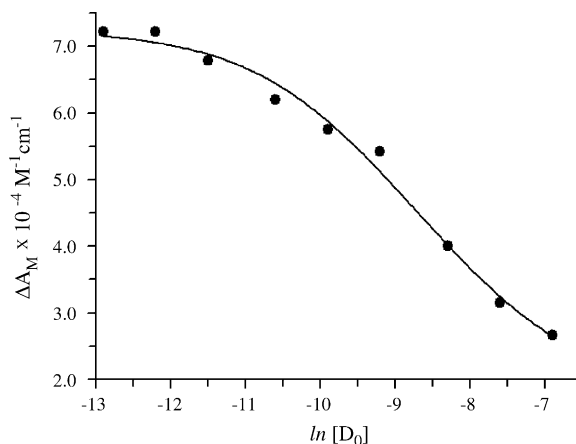

Fig. 2. Dependence of the molar absorption of Methylene Blue in aqueous solution ($\lambda_{max} = 664$ nm) on the logarithm of the dye concentration (smoothed curve).

[14]:

$$\Delta A_M = \varepsilon_d + (\varepsilon_d - \varepsilon_m) \frac{1 - \sqrt{1 + 8\,[D_0] K_d}}{4 K_d [D_0]} \tag{2}$$
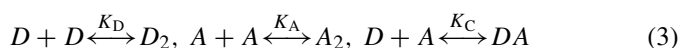
where ε_m and ε_d are extinction coefficients of the dye in monomer and dimer forms. Numeric procedure of fitting the experimental data (Fig. 2) using Eq. (2) was carried out by means of Marquardt algorithm. Calculations resulted in an averaged magnitude of equilibrium constant of MB molecular dimerization: $K_d = 3900 \pm 800$ l/mol, which is in good agreement with the results obtained in [14] in similar solution conditions. The equilibrium constant of CAF dimerization under the given experimental conditions was taken as $K = 14$ l/mol ($T = 293$ K) [15]. Numeric values of the dimerization constants and extinction coefficients of the dye [$\varepsilon_m = 73460 \pm 820$ l/(mol cm), $\varepsilon_d = 8320 \pm 2080$ l/(mol cm)] were calculated on the wavelength of 664 nm and were further used for the hetero-association analysis of MB and CAF in the mixed solution.

3.2. Hetero-association of Methylene Blue and Caffeine in aqueous solution

Experimental spectra of MB in the mixture with CAF at $T = 293$ K are shown in Fig. 3. It is seen that MB monomer region undergoes a bathochromic shift with a simultaneous increase in absorption intensity on increasing the concentration, which is indicative of the hetero-association process in aqueous solution [7]. On the wavelength of 630 nm, an isobestic point is observed characterizing a two-component equilibrium in solution.

The hetero-association analysis of MB and CAF in the mixed solution was made from the concentration dependence of the molar absorption coefficient of MB on the wavelength, corresponding to the absorption maximum of a dye monomer (Fig. 4).

The equilibrium in solution was modeled by the reaction scheme in which the formation of the dimer complexes and 1:1 hetero-complex of the investigated molecules were taken into consideration:

$$D + D \overset{K_D}{\longleftrightarrow} D_2, \ A + A \overset{K_A}{\longleftrightarrow} A_2, \ D + A \overset{K_C}{\longleftrightarrow} DA \tag{3}$$