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FLUORESCENCE CHARACTERISTICS OF FLUORESCEIN AND EOSIN Y SOLUTIONS IN WATER-MICELLAR SURFACTANT MEDIA

The effect of cationic, anionic and nonionic surfactants on the fluorescence properties of fluorescein and eosin Y aqueous solutions has been investigated. It has been found that sodium dodecyl sulfate does not affect the position of the maximum wavelengths of solutions of fluorescein and eosin Y in the study of the effect of an anionic surfactant on the fluorescence emission intensity of dyes. The intensity of the signal of the dye solutions when changing the concentration of anionic surfactant changes little. As the concentration of non-ionic surfactant increases, the fluorescence emission intensity of the fluorescein solutions decreases. In contrast, with increasing concentration of Triton X-100, there is an increase in the signal intensity of solutions of more hydrophobic eosin Y with subsequent access to the "plateau" at C_{TX-100}≥5.1 · 10^{-2} mol/L. The position of the maxima fluorescence emission wavelengths for the fluorescein solutions in the 0-1.0 · 10^{-5} mol/L range of concentrations of cationic surfactant cetylpyridinium chloride remain unchanged. The position of the maxima shifts to the long-wavelength region of the spectrum at higher concentrations of CPC. The nature of the position dependence of the fluorescence emission maxima of eosin Y solutions in the presence of cationic surfactants is similar. The effect of fluorescence quenching has also been shown in the study of the influence of organic substances of cationic nature on the signal intensity of fluorescein solutions. It has been shown that the concentration dependence is linear in the 1.0·10^{-2}–4.0 · 10^{-1}mol/L range of isoniazid molecule concentrations. The data obtained can be implemented in the development of conditions and methods for the determination of pharmacologically active substances of cationic nature by reaction with fluorescein in medicines.

Keywords: fluorescence, surfactant, fluorescein, eosin Y.

Introduction. Luminescence methods are widely used in solving various scientific and applied problems in the fields of chemistry, physics, biology, environmental monitoring and medical diagnostics [1]. The advantages of the luminescence method are the combination of high sensitivity and expressiveness of determination with the possibility of non-destructive control of biological environments and objects [2]. In recent years, surfactants have been increasingly used as supramolecular media for analytical reactions. Advantages, regularities, and methods of rational use of such systems have been studied in detail for spectrophotometry [3]. At the same time, it is known [4] that the use of supramolecular solutions based on surfactants in luminescence methods contribute to the increase of the quantum yield of fluorescence and, accordingly, to the reduction of the detection limit of different analytes [5].

Modification of organic reagents in premicellar surfactant solutions due to association processes [6] is usually singled out when discussing the chemistry of surfactants. On the other hand, the effects associated with the course of solubilization processes at surfactant concentrations above the critical micelle concentration [7] are also distinguished. Fluorescent reagents of different nature, charge, and hydrophobicity are used in the determination of surfactants. Thus, the effect of fluorescence quenching in the fluorescein-cetylpyridinium chloride system in the screening of lipopeptide-producing strains of *Bacillus sp*. used in [8]. The effect of surfactants of different types on the eosin Y fluorescent properties is shown in [9]. The use of surfactant-based systems often leads to improved quality of analytical signal not only in the methods of molecular spectroscopy. For example, the authors [10] attempted to remove color from wastewater containing eosin dye using cloud point extraction in the presence of nonionic Triton X-100.

Fluorescent reagents eosin Y and fluorescein are widely used in spectrophotometric [11, 12], colorimetric [13, 14] and fluorescence assays [15, 16], as well as in the study of the nature of surfactant micelles [17, 18].

The influence of surfactants of different types and their hydrophobicity on the fluorescence characteristics of fluorescein and eosin Y solutions has been investigated in the paper.

Reagents and methods. Widely used anionic xanthene reagents fluorescein and eosin Y are used in the work as fluorescent reagents (R). Cetylpyridinium chloride (CPC) was used as the cationic surfactant. Ethonium, isoniazid, and decamethoxine were selected from the analogs of cationic surfactants. Sodium dodecyl sulfate (SDS) was used as the anionic surfactant. The non-ionic surfactant Triton X-100 (TX-100) was used to create an organized environment. The reagents and surfactants used were "Merck" and "Reachem". Solutions of reagents and surfactants were prepared by dissolving the exact samples in distilled water.

Fluorescence measurements were performed with a Perkin Elmer LS55 fluorescence spectrometer. The pH was controlled by the pH 340-meter with an ESL-43-07 glass electrode.

Results and discussion. In the study of the effect of acidity on the fluorescence characteristics of dyes, it was found that for aqueous fluorescein solutions and in the presence of cationic and anionic surfactants, the position of the emission wavelength maxima (λ_{max}) varies little in the pH=1-12 range and λ_{max}≈516 nm. The shift of λ_{max} to the long-wavelength region of the spectrum is observed in the presence of TX-100 and the position of the maximum wavelengths in the whole pH range is 520 nm (Fig. 1).

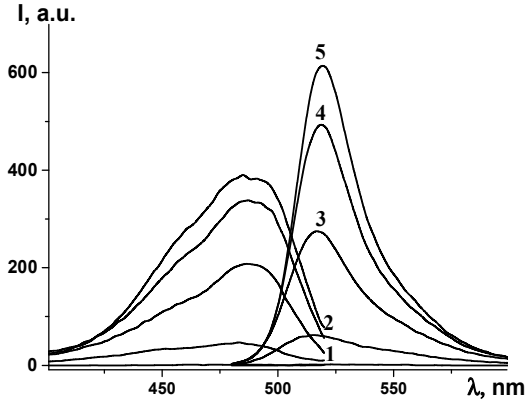

Fig. 1. Fluorescence excitation (540 nm em.) and emission (460 nm exc.) spectra of aqueous solutions of fluorescein in the presence of TX-100 at different pH values. C_R=1.0·10^{-5} mol/L, C_{TX-100}=3.4 · 10^{-2} mol/L. 1: pH=3, 2: pH=6, 3: pH=7, 4: pH=8, 5: pH=11

The positions of emission maxima in the whole pH range do not change significantly and λ_{max}≈538 nm for eosin Y aqueous solutions and in the presence of anionic surfactant. The positions of the fluorescence peaks are shifted to the long-wavelength region of the spectrum in the presence of non-ionic surfactant TX-100. The emission maximum is observed at λ≈531 nm when the dye interacts with the cationic surfactant.

It was found that the fluorescence emission intensity of fluorescein solutions in the presence of surfactants is minimal in the pH=1-3 range. The signal intensity increases at pH=4-9 and reaches a "plateau" at pH>9.

The fluorescence emission intensity of eosin Y solutions and such solutions in the presence of surfactants increases in the pH=2-6 range with the further reaching of the "plateau". The maximum signal intensity is observed at pH=8-12.

Based on the data obtained, further studies of fluorescein-based systems were carried out at pH=11.0 and eosin Y solutions at pH=10.0. The reagents are in solutions in the form of dianions under these conditions.

It was found that sodium dodecyl sulfate does not affect the position of the emission maxima of fluorescein and eosin Y solutions in the study of the effect of an anionic surfactant on the fluorescence emission intensity of dyes. The intensity of the signal of dye solutions when changing the concentration of anionic surfactant changes little.

The position of emission maxima of fluorescein solutions is shifted to the long-wavelength region of the spectrum and λ_{max}≈522 nm at $C_{TX\text{-}100}>5.1\cdot10^{-2}$ mol/L in the presence of non-ionic surfactant in the $0\text{-}1.7\cdot10^{-2}$ mol/L concentration range (curve 1 in Fig. 2a). The shift of λ_{max} of eosin Y solutions into the long-wavelength region of the spectrum is observed at a concentration of TX-100 greater than $1.7\cdot10^{-3}$ mol/L. The position of the maximum fluorescence emission reaches a value of 549 nm at $C_{TX\text{-}100}\geq8.6\cdot10^{-3}$ mol/L (curve 1 in Fig. 2b).

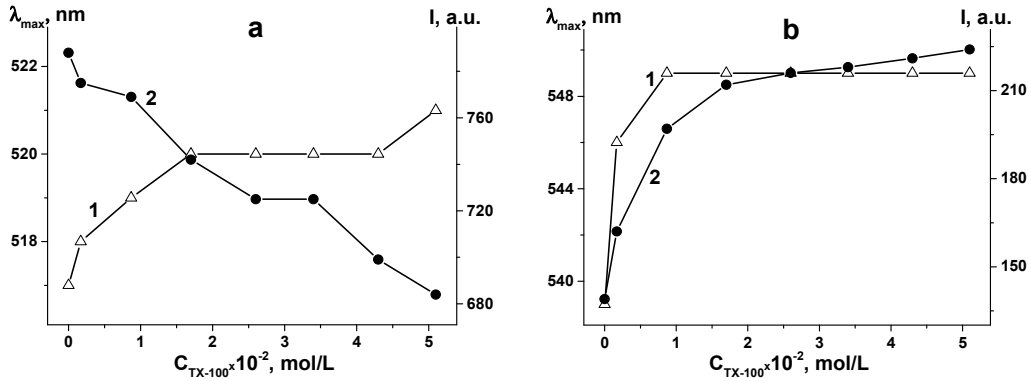

Fig. 2. Dependence of position of the maximum wavelength (1) and intensity (2) of fluorescence emission of fluorescein (a) and eosin Y (b) aqueous solutions on the concentration of TX-100. $C_R=1.0\cdot10^{-5}$ mol/L; pH=11.0 (a), 10.0 (b)

It was found that a decrease in the fluorescence emission intensity of fluorescein solutions was observed with increasing concentration of non-ionic surfactant (curve 2 in Fig. 2a). In contrast, an increase in the signal intensity of solutions of more hydrophobic eosin Y was followed by an increase in the concentration of TX-100 with reaching a "plateau" at $C_{TX\text{-}100}\geq5.1\cdot10^{-2}$ mol/L (curve 2 in Fig. 2b).

The position of the maxima of fluorescence emission wavelengths for the fluorescein solutions in the $0\text{-}1.0\cdot10^{-5}$ mol/L concentration range of CPC remains unchanged and λ_{max}≈517 nm. The position of the maxima shifts to the long-wavelength region of the spectrum at high concentrations of cationic surfactant (curve 1in Fig. 3a). The nature of the $\lambda_{max}=f(C_{CPC})$ dependence for eosin Y solutions is similar (curve 1 in Fig. 3b).

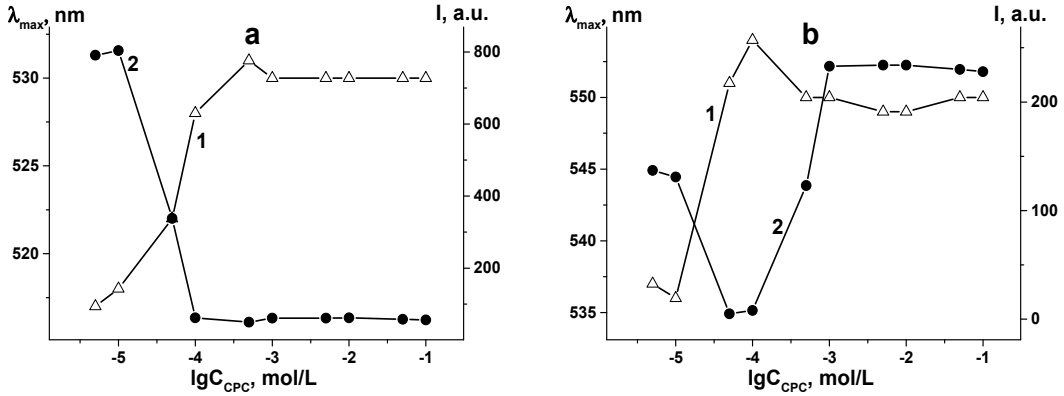

Fig. 3. Dependence of position of the maximum wavelength (1) and intensity (2) of fluorescence emission of fluorescein (a) and eosin Y (b) aqueous solutions on the concentration of CPC. $C_R=1.0\cdot10^{-5}$ mol/L, pH=11.0 (a), 10.0 (б)

The intensity of fluorescence emission of fluorescein solutions in the $0\text{-}5.0\cdot10^{-6}$ mol/L concentration range of cationic surfactant varies little. The signal intensity decreases as the CPC concentration increases. A "plateau" is observed at $C_{CPC}\geq1.0\cdot10^{-4}$ mol/L (curve 2 in Fig. 3a).

The decrease in the fluorescence emission intensity of an aqueous solution of eosin Y is observed when added to cetylpyridinium chloride in the $5.0\cdot10^{-6}\text{-}1.0\cdot10^{-4}$ mol/L concentration range of cationic surfactant. Further increase in the concentration of CPC leads to an increase in the intensity of the signal with the subsequent achievement of the "plateau" at $C_{CPC}=1.0\cdot10^{-3}\text{–}1.0\cdot10^{-1}$ mol/L (curve 2 in Fig. 3b).

Figures 2 and 3 show that the concentration dependences of the λ_{max} position and the fluorescence intensity of surfactants when interacting with reagents are multidirectional. In general, the decrease in signal intensity occurs during the formation of hydrophobic stoichiometric reagent-surfactant associates, which can be explained by the premicellar association of associate-forming particles. Concentration quenching can also cause a decrease in fluorescence intensity. On the other hand, the increase in signal intensity is associated with solubilization processes in the studied systems and changes in the polarity of the microenvironment of the reagent, which is usually accompanied by a λ_{max} bathochromic shift. The different hydrophobicity of eosin Y and fluorescein is also one of the main factors influencing surfactants on the fluorescence intensity of reagent-surfactant systems.

Since the addition of cationic surfactant to fluorescein solutions leads to quenching of fluorescence emission, it was logical to investigate the possibility of determining the organic substances of cationic nature by quenching the fluorescence of the dye.

Ethonium, isoniazid, and decamethoxine have been used as such cationic compounds. Ethonium is an analog of the cationic surfactant and has antiseptic and disinfectant properties [19]. The decamethoxine molecule has a similar structure and medicinal properties [20]. Isoniazid is an active substance in the anti-tuberculosis drug [21]. The volume of isoniazid molecule, compared to ethonium and decamethoxine, is smaller, which may provide better solubilization of the analytic form by surfactant micelles.

It was found that all three substances of cationic nature, similar to cationic surfactant, reduce the intensity of fluorescence emission of fluorescein solutions. Thus, a decrease in the signal intensity of fluorescein solutions is observed at an isoniazid molecule (I-d) concentration greater than $1.0 \cdot 10^{-3}$ mol/L (Fig. 4).

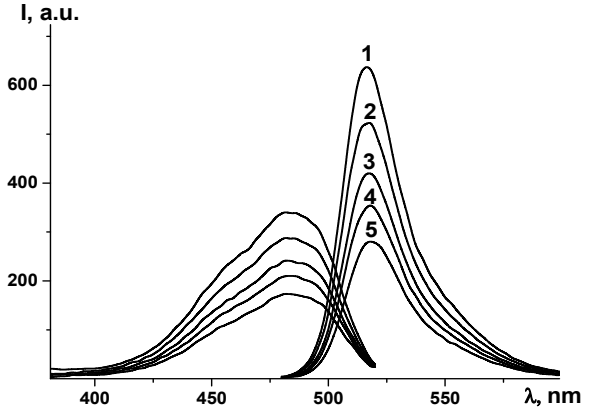

Fig. 4. Fluorescence excitation (540 nm em.) and emission (460 nm exc.) spectra of aqueous solutions of fluorescein in the presence of isoniazid. C_R=1.0·10^{-5} mol/L (1-5), pH=11.0. $C_{I\text{-}d}$, mol/L: 2.0 · 10^{-2} (1), 4.0 · 10^{-2} (2), 6.0 · 10^{-2} (3), 1.0 · 10^{-1} (4)

The position of the maxima of the wavelengths of fluorescence emission of fluorescein solutions increases with increasing isoniazid molecule concentration (curve 1 in Fig. 5). The $I=f(C_{I\text{-}d})$ dependence is linear in the $1.0 \cdot 10^{-2}$- $4.0 \cdot 10^{-1}$ mol/L concentration range of a substance of cationic nature (curve 2 in Fig. 5).

The results obtained can be used to develop conditions and techniques for the determination of organic substances of cationic nature in reaction with fluorescein in medicinal products.

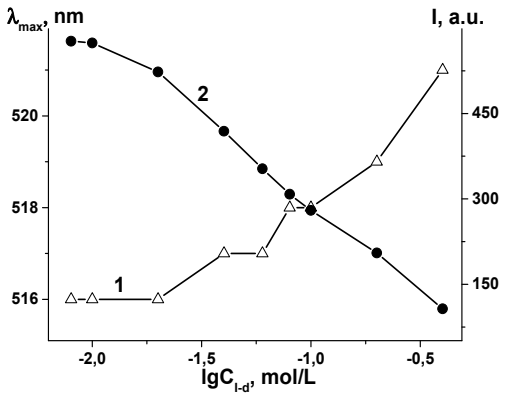

Fig. 5. Dependence of position of the maximum wavelength (1) and intensity (2) of fluorescence emission of fluorescein aqueous solutions on the concentration of isoniazid. C_R=1.0 · 10^{-5} mol/L, pH=11.0

Conclusions. The effect of cationic, anionic and nonionic surfactants on the fluorescence properties of fluorescein and eosin Y aqueous solutions has been investigated. It has been established that anionic surfactant has little effect on the fluorescence emission intensity of dyes. The addition of non-ionic surfactant to fluorescein solutions leads to a decrease in signal intensity. In contrast, an increase in the fluorescence emission intensity of eosin Y solutions is observed when the concentration of TX-100 is increased. It has been revealed that cationic CPC and organic substances of cationic nature cause fluorescence quenching of fluorescein solutions. The data obtained can be implemented in the development of conditions and methods for the determination of pharmacologically active substances of cationic nature by reaction with fluorescein in medicines.

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ФЛУОРЕСЦЕНТНІ ХАРАКТЕРИСТИКИ РОЗЧИНІВ ФЛУОРЕСЦЕЇНУ ТА ЕОЗИНУ Н У ВОДНО-МІЦЕЛЯРНИХ СЕРЕДОВИЩАХ ПАР

Досліджено вплив катіонних, аніонних і неіонних поверхнево-активних речовин (ПАР) на флуоресцентні властивості водних розчинів флуоресцеїну та еозину Н. Під час дослідження впливу аніонної ПАР на інтенсивність емісії флуоресценції барвників встановлено, що ДДСН не вливає на положення максимумів довжин хвиль розчинів флуоресцеїну та еозину Н. Інтенсивність сигналу розчинів барвників у процесі зміни концентрації аніонної ПАР змінюється мало. Встановлено, що під час збільшення концентрації неіонної ПАР спостерігається зменшення інтенсивності емісії флуоресценції розчинів флуоресцеїну. На противагу до цього, прослідковано збільшення інтенсивності сигналу розчинів більш гідрофобного еозину Н у ході збільшення концентрації Triton X-100 із виходом на "плато" за $C_{TX\text{-}100} \geq 5.1 \cdot 10^{-2}$ моль/л. Максимуми довжин хвиль емісії флуоресценції для досліджуваних розчинів флуоресцеїну в інтервалі концентрацій катіонної ПАР ЦПХ 0–1.0 · 10^{-5} моль/л залишаються незмінними. За більших концентрацій ЦПХ положення максимумів зсувається в довгохвильову ділянку спектру. Характер залежності положення максимумів емісії флуоресценції розчинів еозину Н у присутності катіонної ПАР є аналогічним. Під час досліджень впливу органічних речовин катіонної природи на інтенсивність сигналу розчинів флуоресцеїну також показано ефект гасіння емісії флуоресценції. На прикладі ізоніазиду показано, що в діапазоні концентрацій речовини катіонної природи (0.1–4.0) · 10^{-1} моль/л концентраційна залежність має лінійний характер. Отримані в роботі дані можна використати для розробки умов і методик визначення вмісту фармакологічно активних речовин катіонної природи за реакцією із флуоресцеїном у лікарських засобах.

Ключові слова: флуоресценція, поверхнево-активні речовини, флуоресцеїн, еозин Н.

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ФЛУОРЕСЦЕНТНЫЕ ХАРАКТЕРИСТИКИ РАСТВОРОВ ФЛУОРЕСЦЕИНА И ЭОЗИНА Н В ВОДНО-МИЦЕЛЛЯРНЫХ СРЕДАХ ПАВ

Исследовано влияние катионных, анионных и неионных поверхностно-активных веществ (ПАВ) на флуоресцентные свойства водных растворов флуоресцеина и эозина Н. Исследование влияния анионной ПАВ на интенсивность эмиссии флуоресценции красителей показало, что ДДСН не влияет на положение максимумов длин волн растворов флуоресцеина и эозина Н. Интенсивность сигнала растворов красителей при изменении концентрации анионной ПАВ меняется мало. При увеличении концентрации неионной ПАВ наблюдается уменьшение интенсивности эмиссии флуоресценции растворов флуоресцеина. В отличии от этого, установлено увеличение интенсивности сигнала растворов более гидрофобного эозина Н при увеличении концентрации Triton X-100 с выходом на "плато" при $C_{TX\text{-}100} \geq 5.1 \cdot 10^{-2}$ моль/л. Максимумы длин волн эмиссии флуоресценции для исследуемых растворов флуоресцеина в интервале концентраций катионной ПАВ ЦПХ 0–1.0 · 10^{-5} моль/л остаются неизменными. При больших концентрациях катионной ПАВ положение максимумов сдвигается в длинноволновую область спектра. Характер зависимости положения максимумов эмиссии флуоресценции растворов эозина Н в присутствии ЦПХ аналогичный. При исследовании влияния органических веществ катионной природы на интенсивность сигнала растворов флуоресцеина также показано эффект тушения эмиссии флуоресценции. На примере изониазида показано, что концентрационная зависимость имеет линейный характер в диапазоне концентраций вещества катионной природы (0.1–4.0) · 10^{-1} моль/л. Полученные в работе данные могут быть реализованы при разработке условий и методик определения содержания фармакологически активных веществ катионной природы по реакции с флуоресцеином в лекарственных средствах.

Ключевые слова: флуоресценция, поверхностно-активные вещества, флуоресцеин, эозин Н.