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Matching Effects in the Interaction of Ionic Surfactants with Fluorescent Reagents in Micellar Solutions of Triton X-100

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The influence of cationic and anionic surfactant solutions on the character of the fluorescence spectra of reagents of different charge and hydrophobicity in aqueous solutions of nonionic surfactant Triton X-100 has been studied. An increase in the fluorescence intensity and a shift in the position of the fluorescence maximum with increasing hydrophobicity of reagents and ionic surfactants have been shown. The analytical signal of the surfactant is further amplified in the proximity of the charge values of the reagent and the counterion of the surfactant. The non-monotonic nature of the hydrophobicity effect of cationic surfactants on their analytical signal in the system has been shown. The observed effects are explained by the realization of charge and hydrophobic matching in the interaction of surfactants with the fluorescent reagents. The obtained effects are significant in the design of fluorescent systems for the determination and study of surfactant micelles. Conditions for detecting the content of cetylpyridinium chloride by reaction with eosin Y and sodium tetradecyl sulfate by reaction with rhodamine 6G in the presence of Triton X-100 were proposed. The methods have been tested in detecting the content of the ionic surfactants in pharmaceuticals.

Keywords: fluorescent reagent, surfactant, ionic surfactant, associate, solubilization

Aqueous surfactant solutions under certain conditions are ultra-micro-heterogeneous systems (organized media) that arise as a result of the self-organization of diphilic surfactant molecules [1]. This leads to the formation of supramolecular systems (micelles). These supramolecular systems can solubilize molecules and influence intermolecular interactions and photophysical processes in them.2 Organized media based on surfactants in recent years are widely used to modify the analytical signal in various research methods [3, 4]. Cationic, anionic and nonionic surfactants are used to create organized media [5, 6]. Analytical reactions in surfactant solutions are often characterized by improved metrological characteristics [7]. The advantages, patterns and methods of rational use of such systems have studied for molecular spectroscopy methods [8]. In particular, the use of micellar systems in fluorescence analysis makes it possible to increase quantum yields, fluorescence intensity and, accordingly, reduce the detection limits of analytes by changing their photophysical characteristics and the nature of the microenvironment [9].

Modification of reagents in surfactant premicellar solutions due to the association processes are distinguished when discussing the chemistry of surfactant effects [10]. In general, electrostatic and hydrophobic interactions between a reagent particle and a surfactant are always significant in association reactions. On the one hand, associations of organic reagents and surfactants are valuable analytical forms. On the other hand, these associates themselves can act as reagents. Several effects associated with the course of solubilization processes at surfactant concentrations above the critical micelle concentration (CMC) are also noted [11]. Solubilization is an important property of surfactants associated with hydrophobic interactions. In general, the enhancement of electrostatic interactions and the reduction of the role of hydrophobic interactions between associate-forming particles in the transition from aqueous solutions to aqueous-micellar solutions is characteristic of solubilization. The importance of the role of surfactant hydrophobicity and electrostatic interactions in the association of reagents with surfactant counterions is also noted [12, 13]. The direction and magnitude of the change in the nature of the fluorescence maximum wavelength position (λ_{max}) is often explained by the change in the polarity of the microenvironment of the reagent particle due to solubilization [14].

Solutions of nonionic surfactants are valuable media for various chemical reactions. This is due to the great potential for varying the properties of the medium in the microenvironment of molecules, the effects of convergence and concentration of particles, as well as some other factors [15]. Non-ionic surfactant media can significantly change the nature of the reaction processes, even taking up no more than one percent of the solution total volume [11]. Solutions of nonionic surfactants are usually characterized by greater solubilization capacity compared to surfactant solutions of other types. The multidirectional effect of nonionic surfactants on the stability of associates depending on their hydrophobicity is also noted [16]. Nonionic surfactants can stabilize the colloid-chemical state of the system or prevent colloid aggregation in general [17].

Enhancing the stability of the formed associates with the matching (spatial) of the associated particles is an important property of supramolecular systems [18]. This matching is logically associated with the hydrophobicity of the interacting particles in the association reactions of reagents and surfactants [19]. Since the appearance or change of the charge of a particle significantly affects its polar and lyophilic properties, it is logical to connect the matching and strengthening of the stability of particles with the matching of their charges (charge matching). Organized surfactant-based media and the surfactant associations studied with fluorescent reagents are classic objects of supramolecular chemistry. In this regard, studies of the manifestation of supramolecular matching and the enhancement of analytical effects are of considerable interest. The analytical signal in the association reactions usually correlates with the stability of the formed associates.

Ionic surfactants are a relevant and diverse object of determination and analysis. There are several aspects of the determination of ionic surfactants in various environmental objects. First, it is a spectrophotometric determination of microconcentrations of ionic surfactants in environmental objects using general approaches to the extraction of ionic surfactant associates with dye counterions. Secondly, it is the determination of ionic surfactants in technological solutions. Third, it is the quantitative determination of ionic surfactants in pharmaceuticals [20]. According to the documentation within the pharmaceutical industry [21, 22], cationic surfactants in pharmaceuticals are determined by infrared and ultraviolet spectroscopy, spectrophotometry, thin-layer chromatography, and titration [23]. The European Pharmacopoeia and the British Pharmacopoeia use the method of gas chromatography to determine anionic surfactants in pharmaceuticals. According to the State Pharmacopoeia of Ukraine, quantitative determination of anionic surfactants is performed by titration. Electrochemical methods [24, 25], chromatographic methods [26, 27], and molecular spectroscopy analysis [28-31] are the most commonly used methods for the determination of ionic surfactants in pharmaceuticals in general laboratory practice.

The complexity and lack of sensitivity of the known techniques do not allow to quickly control the content of ionic surfactants in places of their local application. The fluorescence method is actively used in medicine, physics, biology, food analysis, and pharmaceutical research. The method is characterized by high sensitivity and specificity. The fluorescence is more approachable compared to chromatographic methods. Therefore, the choice of fluorescence to determine the content of ionic surfactants in pharmaceuticals is quite logical and competitive.

The work aimed to investigate the influence of the main factors on the fluorescent signal of the ionic surfactants in the association reactions, search for manifestations of matching effects, and development of rational conditions for fluorescent determination of the ionic surfactants in the pharmaceuticals. The subject of the study is the analytical signal of the ionic surfactants in their interaction with reagents in the environment of nonionic surfactants Triton X-100.

Experimental sections

Cationic dyes luminol (Lum), lucigenin (Luc), rhodamine 6G (R6G) and rhodamine B (RB) and anionic dyes fluorescein (Flu), eosin Y (EY) and morin (Mor) were from Reachem (Russia). Dyes were used as fluorescent reagents (R). The choice of reagents was due to the need to vary the charge of the reagent particle in surfactant solutions. Alkylpyridinium chlorides with carbon numbers (n) in hydrocarbon radical 11-16 such as 1-undecylpyridin-1-ium chloride (C_{11}), 1-dodecylpyridin-1-ium chloride (C_{12}), 1-tridecylpyridin-1-ium chloride (C_{13}), 1-tetradecyl-pyridin-1-ium chloride (C_{14}), 1-pentadecyl-pyridin-1-ium chloride (C_{15}) and 1-hexa-decylpyridin-1-ium chloride (C_{16}, cetylpyridinium chloride, CPC) were used as cationic surfactants (c-Surf). Used cationic surfactants were from Sigma-Aldrich (Germany) of ≥99% purity. Sodium dodecyl sulfate (SDS) and sodium tetradecyl sulfate (STS) were used as anionic surfactants. Used anionic surfactants were from Sigma-Aldrich (Germany) of ≥99 % purity. Triton X-100 (TX-100) of laboratory grade (Merck, USA) was used as a nonionic surfactant. Solutions of dyes and surfactants (Surf) 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.

The hydrophobicity of the reagent molecules/ions was transmitted through the octanol-water partition coefficient (lgD_R) [13]. lgD_R makes it possible to assess the hydrophobicity of the particle in real conditions, taking into account the form of its existence at certain pH values.

The change in the nature of the reagent solutions fluorescence spectra when interacting with surfactants was considered as the appearance of the surfactant corresponding analytical signal. The ΔI and Δλ parameters were chosen as the most significant parameters of the analytical signal quality. ΔI is the difference in the fluorescence intensity of the analytical system in the absence and the presence of the analyte (surfactant) at the fluorescence maximum wavelength position. Δλ is the change in the λ_{max} position of the analytical system in the absence and the presence of surfactants. The value of the ΔI parameter, among other factors, logically depends on the fluorescence intensity of the reagent itself. Therefore, the comparison of the behavior of different reagents in surfactant solutions should be discussed using the normalized intensity of the analytical signal

$\Delta I/I_R$, where I_R is the fluorescence emission intensity of the individual reagent solution. The $\Delta I/I_R$ parameter can be both positive and negative. Positive values of this parameter are more valuable in the analysis, as their measurement involves a smaller value of the signal of the comparison solution, which is a solution of the reagent compared to the signal in the presence of surfactants. Negative values of $\Delta I/I_R$ can also be used for analytical measurements. However, negative values of the normalized intensity of the analytical signal indicate a larger signal of the comparison solution and, consequently, a potential increase in measurement errors.

The charges (Z) of the reagent particles at optimized pH values, lgD_R, $\Delta I/I_R$ and $\Delta\lambda$ of reagents individual solutions and aqueous systems of reagent-ionic surfactant, respectively, are given in Table 1.

Results and discussion

The addition of the surfactant to aqueous reagent solutions can lead to changes in the fluorescence intensity and the position of the excitation and fluorescence maxima of the reagents. Surfactant concentration dependences for the position of the maximum fluorescence wavelengths of reagents in aqueous solutions and micellar solutions of nonionic surfactant TX-100 have investigated. This approach has been applied to all possible combinations of investigated reagents and used surfactants.

The influence of the concentration of cationic CPC on the nature of the fluorescence spectra has considered on the example of anionic fluorescein, Fig. 1. Thus, $\lambda_{max} \approx 518$ nm for an aqueous solution of fluorescein and in the $0\text{-}1.0\cdot10^{-5}$ M concentration range of CPC (curve 1 in Fig. 1). Bathochromic shift is observed with increasing concentration of CPC (curve 2 in Fig. 1). λ_{max} is 522 nm when adding nonionic TX-100 to a solution of fluorescein at $C_{TX\text{-}100} \geq 6.0\cdot10^{-2}$ M (curve 3 in Fig. 1). The position of λ_{max} for R-CPC-TX-100 three-component systems at C_{CPC} = 0-$5.0\cdot10^{-4}$ M changes little and $\lambda_{max} \approx 519$ nm. A further increase in the concentration of cationic surfactant leads to a bathochromic shift (curve 4 in Fig. 1).

The magnitude of the reagent bathochromic shift in surfactant micellar solutions correlates with a decrease in the polarity of the microenvironment of the reagent in the solubilized state in comparison with that in aqueous solutions.

The surfactant-concentration dependences of the change in the fluorescence intensity of reagents in aqueous solutions and TX-100 micellar solutions have also investigated. Such signal intensity studies have performed for all possible combinations of reagents and surfactants used.

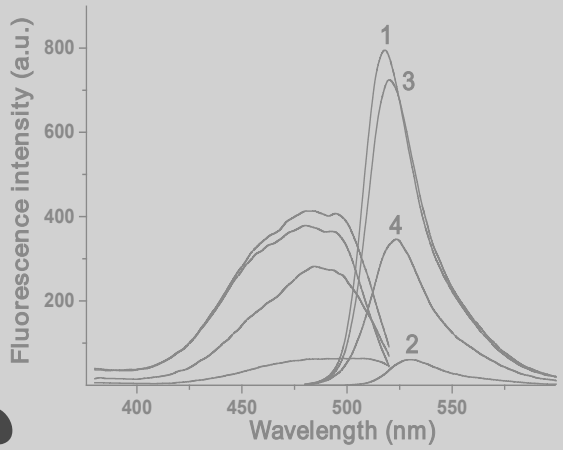

Figure 1. Excitation and fluorescence spectra of fluorescein solutions in the presence of surfactants. $C_R = 1.0\cdot10^{-5}$ M (1-4), $C_{CPC} = 1.0\cdot10^{-3}$ M (2, 4), $C_{TX\text{-}100} = 3.4\cdot10^{-2}$ M (2, 3), pH = 11. 1: R-water, 2: R-CPC, 3: R-TX-100, 4: R-CPC-TX-100.

Table 1. Normalized fluorescent analytical signal of ionic surfactant in reaction with reagents and $\Delta\lambda$ in aqueous solutions. $C_R = 1.0\cdot10^{-5}$ M, $C_{Surf} = 5.0\cdot10^{-2}$ M.

R	Mor	Flu	EY	Lum	RB	R6G
Z[a]	-4	-2	-2	0	+1	+2
lgD_R [b]	-4.89	-0.76	2.21	0.33	1.99	3.12
			R-CPC			
$\Delta I / I_R$	-1	-0.93	0.65	-0.67	-0.27	0.27
$\Delta\lambda$, nm	0	12	11	0	1	1
			R-SDS			
$\Delta I / I_R$	0	-0.01	0.06	-0.19	-0.16	1.16
$\Delta\lambda$, nm	3	0	0	0	8	0

[a] Z was determined considering the available in the literature values of pK or estimates of protolytic constants calculated by the program ICDLab v.6.0.

[b] The lgD_R values were taken from databases [32, 33] and in the absence of such information were calculated by the program ICDLab v.6.0.

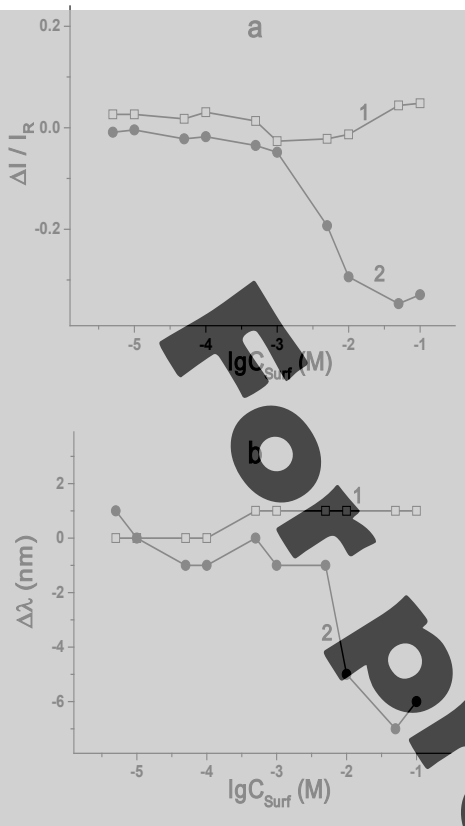

Figure 2. Concentration dependence of the normalized analytical signal (a) and the change in the λ_{max} position (b) of the fluorescence of eosin Y solutions on the concentrations of CPC (1) and SDS (2) in the presence of TX-100. $C_R = 1.0 \cdot 10^{-5}$ M, $C_{TX-100} = 3.4 \cdot 10^{-2}$ M, pH = 10.

Surfactant concentration dependences for ΔI and Δλ have considered on the example of anionic eosin Y. Thus, ΔI and Δλ parameters change little when the cationic surfactant is added to the eosin Y solution in the presence of nonionic TX-100 (curves 1 in Figs. 2a and 2b). The signal intensity also changes little when anionic SDS is added to the reagent aqueous-micellar solution at $C_{SDS} < CMC$ [34]. The value of ΔI of the R-SDS associate decreases at $C_{SDS} \approx CMC$ (curve 2 in Fig. 2a). A similar effect is observed on the $\Delta\lambda = f(C_{SDS})$ dependence (curve 2 in Fig. 2b).

The study of the effect of ionic surfactants micellar concentrations on the normalized analytical signal and Δλ in the presence of nonionic TX-100 has performed for reagents of different charges. For cationic CPC (Fig. 3a) is shown that all values of $\Delta I/I_R$ (except the system EY-CPC-TX-100) are negative. The normalized analytical signal of the CPC increases with the transition from negative to positive charge of the reagents. The analytical signal of the CPC for RB^+ is close to zero. The positive value of the normalized analytical signal is characteristic of the EY^{2-}, which is explained by the highest value of lgD_{EY} among the studied anionic reagents. Such lgD_R large value provides a different nature of the microenvironment of the EY-CPC solubilized associate.

The value of Δλ for all studied systems R-CPC-TX-100 is positive (Fig. 3b). The highest value of Δλ is observed for the Flu^{2-}, i.e., in the proximity of charges of associating oppositely charged particles under conditions conducive to the formation of electroneutral associates.

The example of anionic SDS (Fig. 3c) shows that the interaction of negatively charged reagents with anionic surfactant registered both negative and positive $\Delta I/I_R$ values. Thus, the maximum negative value of $\Delta I/I_R$ is observed for the EY^{2-}. The highest positive value of the normalized analytical signal among the anionic reagents is registered for the Flu^{2-}. The charge of fluorescein is close to the charge of the surfactant counterion, which is a condition for approaching the charge matching. For electroneutral and positively charged reagents, under conditions of electrostatic repulsion of reagent particles and surfactants, positive values of $\Delta I/I_R$ are characteristic. The luminol-SDS-TX-100 system is characterized by the highest positive value of $\Delta I/I_R$.

The nature of the $\Delta\lambda = f(Z)$ dependence (Fig. 3d) is similar to the nature of the $\Delta I/I_R = f(Z)$ dependence (Fig. 3c) for the studied systems. Thus, both negative and positive values of Δλ have registered. The maximum negative values of Δλ are observed when interacting with anionic SDS in solutions of fluorescein and eosin Y. The maximum positive values of Δλ are observed for rhodamine 6G, i.e., for the most hydrophobic among the studied reagent under conditions conducive to the neutralization of its charges. The manifestation of the effect of changing the polarity of the reagent microenvironment under conditions of weak hydrophobic solvation for the same charged reagent particles and surfactants is registered for Mor^{4-}.

Thus, the increase in the fluorescence intensity and the shift of the λ_{max} position with increasing contribution of the role of electrostatic interactions between the reagent particle and the ionic surfactant is shown in Fig. 3. At the same time, the analytical signal of the surfactant is further amplified in the proximity of the opposite charges of the reagent and the ionic surfactant.

The effect of the reagent hydrophobicity on the parameters of the analytical signal has monitored using the octanol-water partition coefficient. It has considered expedient to compare the analytical signal of reagents of different charges when interacting with ionic surfactants on the $\Delta I/I_R = f(lgD_R)$ and $\Delta\lambda = f(lgD_R)$ dependences.

The effect of micellar concentrations of the nonionic TX-100 on the fluorescent properties of individual reagent solutions was interesting to trace before studying the effect of ionic surfactants. The $\Delta I/I_R = f(lgD_R)$ dependence for TX-100 is characterized by a pronounced maximum at the value of $lgD_R \approx 2$

Figure 3. The dependence of $\Delta I/I_R$ (a, c) and $\Delta\lambda$ (b, d) of the CPC (a, b) and SDS (c, d) in the reaction with the reagents on the reagent charge in the presence of TX-100. $C_R = 1.0 \cdot 10^{-5}$ M, $C_{CPC, SDS} = 5.0 \cdot 10^{-2}$ M, $C_{TX-100} = 3.4 \cdot 10^{-2}$ M.

for rhodamine B (Fig. 4). This lgD_R value is close to the calculated values of TX-100 lgD (lgD_{TX-100}). Thus, the calculated lgD_{TX-100} value for the 9 degree of oxyethylation is 2.02, and for the 10 degree of oxyethylation is 1.67. In other words, the TX-100 maximum analytical signal is observed for reagents with lgD_R values close to the lgD value of TX-100 itself and may indicate the appearance of hydrophobic matching. The $\Delta I/I_R$ value is positive in almost the entire range of lgD_R values. It indicates the predominance of solubilization effects. It is noteworthy that a negative $\Delta I/I_R$ value is registered for an uncharged luminol particle (under conditions of the charge matching). The $\Delta\lambda = f(lgD_R)$ dependence for TX-100 is also characterized by a clear maximum at $lgD_R > 2$ and minimum $\Delta\lambda$ values at $lgD_R \approx 0\text{-}2$.

The $\Delta I/I_R = f(lgD_R)$ dependence for cationic CPC is also characterized by the presence of a maximum similar to the TX-100 effect (curve 1 in Fig. 5). Herewith the λ_{max} position for CPC, relative to that for TX-100, is shifted to higher values of the reagent hydrophobicity. Thus, the maximum $\Delta I/I_R$ value in CPC systems in the absence of TX-100 was registered for eosin Y ($lgD_R = 2.21$). The $\Delta I/I_R$ value for reagents with $lgD_R \leq 2$ is negative. The maximum positive $\Delta I/I_R$ values are characteristic of reagents with $lgD_R > 2$. The positions of the extremums for R-CPC two- and R-CPC-TX-100 three-component systems coincide (curve 2 in Fig. 5). However, all $\Delta I/I_R$ values, except the maximum, for the three-component system are negative, and the actual maximum value is less than that in the absence of TX-100. That is, the leveling of the particles hydrophobicity effect on the analytical signal during their association in solutions of nonionic surfactants is observed. The nature of the $\Delta\lambda = f(lgD_R)$ dependences for CPC is similar to the nature of the $\Delta I/I_R = f(lgD_R)$ dependence.

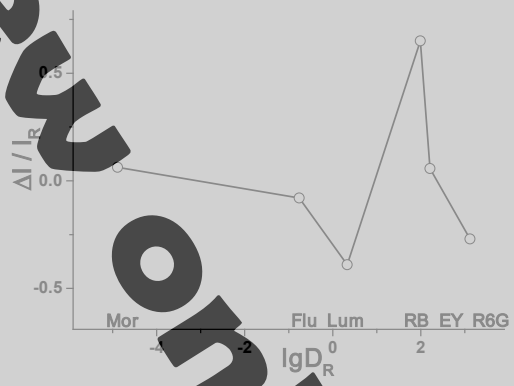

Figure 4. Dependence of the TX-100 normalized analytical signal in the reaction with reagents on lgD_R. $C_R = 1.0 \cdot 10^{-5}$ M, $C_{TX-100} = 3.4 \cdot 10^{-2}$ M.

The normalized analytical signal of anionic SDS in two-component systems with dyes in the absence of TX-100 when changing the hydrophobicity of the reagent changes little (Table 1). A significant positive $\Delta I/I_R$ value was registered only for highly hydrophobic $R6G^{2+}$. It is due to the combination of electrostatic attraction of the dye cation and surfactant anion enhanced by hydrophobic interactions in the system.

On the other hand, the $\Delta I/I_R = f(lgD_R)$ dependence for SDS in the three-component systems is characterized by the presence of a positive maximum for luminol. The $\Delta\lambda = f(lgD_R)$ dependence for SDS in two-component systems is characterized by the presence of the minimum at $lgD_R = 2.21$. The maximum at $lgD_R = 1.99$ on the $\Delta\lambda = f(lgD_R)$ dependence is registered in the systems R-SDS-TX-100. This maximum correlates with the values of both lgD_{TX-100} and is close to the lgD value of SDS (lgD_{SDS}). Thus, $lgD_{SDS} = 1.60$ [35] and $lgD_{SDS} = 1.89$ calculated by the ICDLab v.6.0. program.

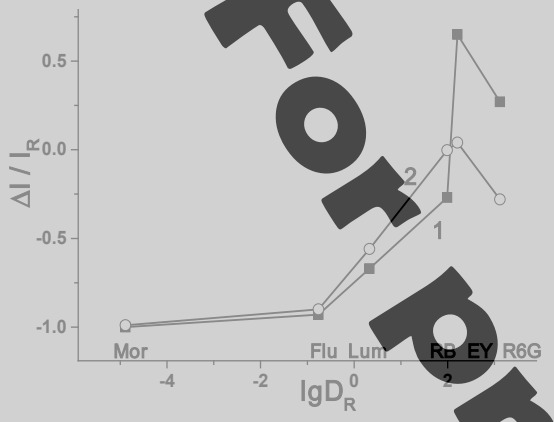

Figure 5. Dependence of the CPC normalized analytical signal in the reaction with reagents in the absence (1) and the presence (2) of TX-100 on lgD_R reagents. $C_R = 1.0 \cdot 10^{-5}$ M, $C_{CPC} = 5.0 \cdot 10^{-2}$ M, $C_{TX-100} = 3.4 \cdot 10^{-2}$ M.

It should be noted that the matching effects and amplification of the analytical signal in classical supramolecular systems of the «host-guest» type are usually observed on the two types dependences [36, 37]. First, it is a type of dependence of the study of the system analytical signal when changing the size of the guest at a fixed size of the host/receptor. Secondly, this is a type of similar signal dependences on the sizes of the guest/receptor at the fixed sizes of the guest. Therefore, the influence of the length of the cationic surfactant hydrocarbon chain on the fluorescent properties of the R-c-Surf and R-c-Surf-TX-100 associates at $C_{c\text{-}Surf} > CMC$ together with the effect of the reagent hydrophobicity have investigated. Such studies have performed for all reagents studied.

Thus, the $\Delta I/I_R = f(n)$ dependence for eosin Y systems passes through a small minimum for c-Surf with n=12 (curve 1 in Fig. 6). The $\Delta I/I_R$ value goes to the «plateau» with a subsequent increase in n. The $\Delta I/I_R$ values change to positive with increasing hydrophobicity of cationic surfactant. It is typical of systems that provide effective hydrophobic solvation of the reagent. On the other hand, the maximum on the $\Delta\lambda = f(n)$ dependence is observed for c-Surf with n=12. This maximum can be interpreted as the implementation of the conditions of matching between the interacting particles.

In general, the nature of the $\Delta I/I_R = f(n)$ and $\Delta\lambda = f(n)$ dependences differs for reagents of different hydrophobicity and charge. Thus, the $\Delta I/I_R$ values for rhodamine B aqueous solutions increase monotonically with increasing n. On the other hand, the changes in the normalized analytical signal of c-Surf for rhodamine 6G solutions are small. Additionally, the dependences of the λ_{max} position on n in the fluorescence spectra of RB^{1+} and $R6G^{2+}$ aqueous solutions were not registered. A large number of factors that affect the $\Delta I/I_R$ and $\Delta\lambda$ values of causes this effect. It also emphasizes the importance of inflections and extrema registered on the $\Delta I/I_R = f(n)$ and $\Delta\lambda = f(n)$ dependences for other reagents.

The leveling effect of nonionic surfactant micellar concentrations on the fluorescent characteristics of R-c-Surf associates of different hydrophobicity has established. Thus, the $\Delta I/I_R$ and $\Delta\lambda$ values for associates with cationic surfactants of different hydrophobicity in TX-100 medium change little. On the one hand, the detected leveling effect of TX-100 reduces the selectivity of the determination of cations of a certain hydrophobicity. On the other hand, this phenomenon expands the range of detectable cationic organic substances by the fluorescent method. The association of reagents with surfactant counterions in pure aqueous solutions usually makes it possible to implement methods for determining only the most hydrophobic cationic compounds. R-c-Surf associates modified with nonionic surfactant expand the possibilities of creating methods for the determination of short-chain cationic surfactants and other classical quaternary ammonium salts. An additional advantage of such systems in the analysis is the simplification of the construction of calibration dependences and the solution of the problem of choosing a standard surfactant.

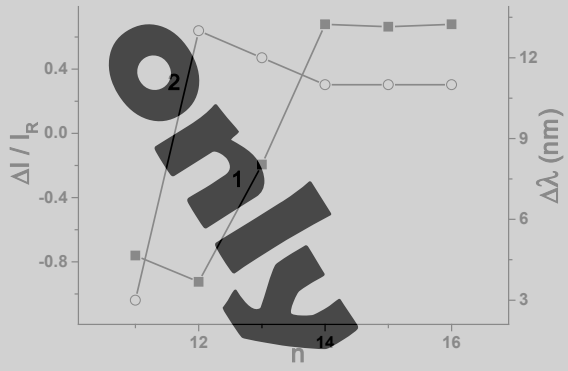

Figure 6. Dependence of $\Delta I/I_R$ (1) and $\Delta\lambda$ (2) of aqueous solutions of eosin Y on the cationic surfactants hydrocarbon chain n. $C_R = 1.0 \cdot 10^{-5}$ M, $C_{c\text{-}Surf} = 1.0 \cdot 10^{-3}$ M, $C_{TX-100} = 3.4 \cdot 10^{-2}$ M.

The revealed regularities showed the expediency of using three-component reagent-ionic surfactant-TX-100 systems in the design of analytical systems for the determination of cationic and anionic surfactants by the fluorescent method. Thus, eosin Y was selected for the determination of cationic cetylpyridinium chloride and rhodamine 6G was taken for the determination of anionic sodium tetradecyl sulfate. The choice of anionic eosin Y and cationic rhodamine 6G is a compromise of the analytical capabilities of the reagents to determine the appropriate surfactant counterions, taking into account the discussed effects of charge and hydrophobic matching between associate-forming particles. The choice of concentrations of the nonionic surfactant in the proposed conditions for the determination of the ionic surfactants also represents a compromise of its stabilizing action and the effect of TX-100 on the analytical signal value of the ionic surfactants. Optimal conditions for the determination of the ionic surfactants in the reaction with fluorescent reagents are in the context of previous studies [38, 39].

The linearity of the calibration dependence of fluorescence detection of cetylpyridinium chloride with eosin Y in the presence of TX-100 (Table 2) is 0-2.0·10^{-5} M (0-7.2 mg L^{-1}). The values of LOD (3σ-criterion) and LOQ (10σ-criterion) were calculated based on the parameters of the calibration curve and were 0.3 mg L^{-1} and 1.1 mg L^{-1}, respectively.

The linearity of the calibration dependence of fluorescence detection of sodium tetradecyl sulfate with rhodamine 6G in the presence of TX-100 (Table 3) is 0-1.0·10^{-5} M (0-3.2 mg L^{-1}). The calculated values of LOD and LOQ were 0.2 mg L^{-1} and 0.7 mg L^{-1}, respectively.

The robustness of the developed methods was tested when assessing the stability of the investigated solutions. Thus, the measurement of the fluorescence intensity of reagent-ionic surfactant solutions at low, medium, and high concentrations (Tables 2 and 3) was performed immediately after the preparation of the respective solutions and 24 hours after the preparation of the solutions. The found values of RSD did not exceed 0.9 %.

The developed conditions were used to determine the content of the ionic surfactants in pharmaceuticals. Determination of cetylpyridinium chloride content was performed in «Septolete Total» lozenges and «Septolete Plus» sprays manufactured by «Krka, d.d.» (Slovenia). 1 «Septolete Total» lozenge contains 1 mg cetylpyridinium chloride and 3 mg benzydamine hydrochloride (excipients: peppermint oil, levomenthol, sucralose, citric acid anhydrous, isomaltit, lemon flavor, honey flavor, curcumin dye). 1 mL of «Septolete Plus» spray contains 2 mg of cetylpyridinium chloride and 10 mg of benzocaine (excipients: ethanol, glycerin, sodium saccharin, peppermint oil, purified water).

Determination of sodium tetradecyl sulfate content was performed in a solution for injection «Fibro-Vein» manufactured by Hameln Pharmaceuticals (Germany). 1 mL of «Fibro-Vein» solution contains 3 mg of sodium tetradecyl sulphate (excipients: benzyl alcohol; sodium hydrogen phosphate, dodecahydrate; potassium dihydrogen phosphate; sodium hydroxide; water for injections).

Determination of cetylpyridinium chloride in lozenges «Septolete Total». Four «Septolete Total» lozenges were dissolved in 100 mL of distilled water. 2.5 mL (corresponding to the content of CPC in the lozenge ≈ 4.0 mg L^{-1}) was taken from the prepared solution and analyzed according to the method: the aliquot volume was placed in a flask with a volume of 25.0 mL, which contained 2.5 mL 1.0·10^{-4} M solution of eosin Y and 1.5 mL of 1.0·10^{-2} M TX-100 solution. Distilled water was made to final volume. The contents of the flasks were mixed thoroughly and the pH was adjusted to 10.0 with NaOH solution.

Table 2. The accuracy and precision of the method of determining CPC in the reaction with eosin Y in the presence of TX-100. C_R=1.0·10^{-5} M, $C_{TX\text{-}100}$=6.0·10^{-4} M, pH=10, n=3, P=0.95.

Introduced, mg L^{-1}	Intra-day			Inter-day		
	Found, mg L^{-1}	S	RSD, %	Found, mg L^{-1}	S	RSD, %
0.4	0.40±0.01	0.003	0.6	0.39±0.01	0.005	1.2
3.6	3.61±0.02	0.01	0.3	3.60±0.02	0.01	0.4
7.1	7.09±0.12	0.07	0.9	7.11±0.07	0.05	0.7

Table 3. The accuracy and precision of the method of determining STS in the reaction with rhodamine 6G in the presence of TX-100. C_R=1.0·10^{-5} M, $C_{TX\text{-}100}$=1.0·10^{-4} M, pH=4, n=3, P=0.95.

Introduced, mg L^{-1}	Intra-day			Inter-day		
	Found, mg L^{-1}	S	RSD, %	Found, mg L^{-1}	S	RSD, %
0.3	0.29±0.01	0.003	0.9	0.30±0.01	0.004	1.2
1.6	1.61±0.01	0.007	0.4	1.60±0.01	0.008	0.5
3.1	3.11±0.03	0.02	0.5	3.09±0.03	0.02	0.7

Determination of cetylpyridinium chloride in «Septolete Plus» spray. 1 mL of «Septolete Plus» spray was diluted with distilled water to a volume of 25.0 mL. 1.0 mL (corresponding to the content of CPC in the solution ≈3.2 mg L^{-1}) was taken from the prepared solution and analyzed according to the method: the aliquot volume was placed in a flask with a volume of 25.0 mL, which contained 2.5 mL $1.0 \cdot 10^{-4}$ M solution of eosin Y and 1.5 mL of $1.0 \cdot 10^{-2}$ M TX-100 solution. Distilled water was made to final volume. The contents of the flasks were mixed thoroughly and the pH was adjusted to 10.0 with NaOH solution. The content of cetylpyridinium chloride in the pharmaceuticals was determined using the appropriate calibration dependence and the method of standard addition.

Determination of sodium tetradecyl sulfate in the solution for injection «Fibro-Vein». 1 ml of «Fibro-Vein» solution was diluted with distilled water to a volume of 100.0 mL. 1.0 mL (corresponding to the content of STS in the solution ≈ 1.2 mg L^{-1}) was taken from the prepared solution and analyzed according to the method: the aliquot was placed in a 25.0 mL flask containing 2.5 mL of $1.0 \cdot 10^{-4}$ M rhodamine 6G solution and 2.5 mL of $1.0 \cdot 10^{-3}$ M TX-100 solution. Distilled water was made to final volume. The contents of the flasks were mixed thoroughly and pH 4.0 was adjusted with HNO_3 solution. The content of sodium tetradecyl sulfate in «Fibro-Vein» solution was determined using the appropriate calibration dependence and the method of standard addition.

Table 4 shows the results of fluorescent detection of ionic surfactants in the pharmaceuticals.

The obtained data make it possible to conclude that the content of the active substance in the studied pharmaceuticals meets the requirements of European Pharmacopoeia.

Conclusions

The fluorescence spectra of reagents in the interaction with the ionic surfactants in aqueous solutions and micellar solutions of nonionic Triton X-100 have studied. The tendencies of influence of charge and hydrophobicity of reagent particles and solubilization effects on spectral changes have investigated. It has shown that the largest changes in the nature of the spectra and, accordingly, the largest analytical signal values of ionic surfactants in the studied two-component systems are recorded at concentrations greater than CMC. The negative value of the CPC normalized analytical signal for three-component systems in the presence of TX-100 increases with increasing particle charge during the transition from anions to cations of reagents. Herewith the maximum value of the analytical signal of ionic surfactants parameters on the dependences $\Delta I/IR = f(Z)$ and $\Delta\lambda = f(Z)$ is registered for the reagents of the corresponding opposite charge. This occurs under conditions that promote the formation of electroneutral associates of oppositely charged reagent particles and ionic surfactants in the presence of TX-100, the interaction of which is enhanced by hydrophobic forces in the system. The appearance of $\Delta I/I_R$ and $\Delta\lambda$ extremes on the analytical signal dependences of the nonionic TX-100, as well as cationic CPC and anionic SDS on the hydrophobicity of the fluorescent reagent, is systematically shown under the conditions of hydrophobic matching of the reagent and surfactant. Such an amplification of the analytical signal of the surfactant has registered for the corresponding dependences on the number of carbon atoms in the hydrocarbon chain of cationic surfactants. The systematic manifestation of the amplification of the analytical signal of ionic surfactants under the conditions of hydrophobic and charge matching with the reagent is explained in the work by the supramolecular nature of the studied systems and the manifestation of effects similar to «host-guest» effects. Nonionic surfactant micellar concentration level the specificity of interactions of fluorescent reagents with surfactant ions of different hydrophobicity. This is due to a decrease in the role of hydrophobic interactions and an increase in the contribution of electrostatic interactions in the formation of the corresponding associations. The obtained results were used in the development of methods for fluorescent detection of the ionic surfactants in pharmaceuticals. The developed method for the determination of cetylpyridinium chloride was tested in assessing the content of the cationic surfactants in the lozenges «Septolete Total» and the «Septolete Plus» spray. Sodium tetradecyl sulfate was determined the solution for injection «Fibro-Vein».

Table 4. The results of fluorescent detection of the active substances in the pharmaceuticals «Septolete Total», «Septolete Plus» and «Fibro-Vein» (n=5, P=0.95).

Pharmaceutical	Declared by the manufacturer[a]	Found according to the developed method
	The content of cetylpyridinium chloride, mg	
«Septolete Total»	1.0±0.1	0.99±0.02
	The content of the active substances, mg mL^{-1}	
«Fibro-Vein»	3.0±0.2	3.06±0.07
«Septolete Plus»	2.0±0.1	1.96±0.05

[a] 5% maximum percentage deviations from the stated content of active substance in pharmaceuticals according to European Pharmacopoeia [22].

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