
PHYSICAL CHEMISTRY
OF SURFACE PHENOMENA

Mixed Micellar Solutions of Hexadecylpiperidinium Surfactants and Tween 80: Aggregation Behavior and Solubilizing Properties

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Received November 5, 2019; revised November 28, 2019; accepted January 21, 2020

Abstract—The aggregation behavior of mixed micellar solutions of cationic hexadecylpiperidinium surfactants and nonionic surfactant Tween 80 is studied. The critical micelle concentration is determined by varying the component ratios, and a negative deviation from the ideal mixing model can be observed (synergistic effect). The adsorption parameters and surface potential of mixed micelles are estimated. The solubilizing effect individual and mixed compositions have on such hydrophobic biologically active compounds as bioflavonoid quercetin and systemic fungicide carboxin is characterized quantitatively via spectrophotometry.

Keywords: mixed micelles, solubilization, quercetin, carboxin

DOI: 10.1134/S0036024420090198

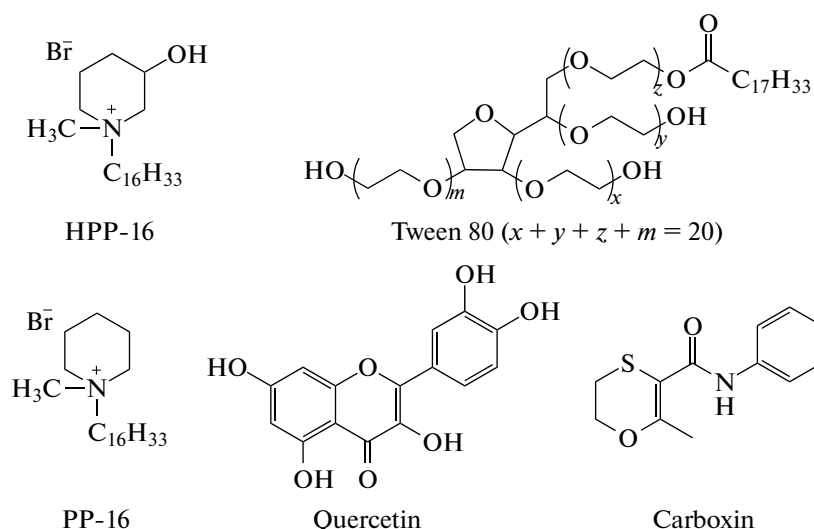
INTRODUCTION

Surfactants are widely used in biotechnology, pharmacology, and agriculture as solubilizers, ways of delivering biologically active substances, and adjuvants that improve the properties of drugs and pesticides [1–4]. They provide better solubility to drugs in aqueous systems, thereby increasing their bioavailability, and improve the surface wetting and transport of target substances into animal and plant cells. Micellar solutions based on low-toxic nonionic surfactants (e.g., Triton-X-100, Tween 20, Tween 80, and amphiphilic block copolymers) make up a considerable proportion of the systems used for these purposes [5–8]. However, these compounds are often less effective than the more toxic cationic surfactants. The latter exhibit a high solubilization effect determined not only by hydrophobic forces but by electrostatic forces as well, and involving other types of interactions, particularly the formation of hydrogen bonds when functional fragments are present in the surfactant molecule [9–11]. The charge of a micelle used as a carrier for an active drug frequently ensures its better contact with

biological surfaces and organisms, thereby increasing its effectiveness [12–14]. The use of binary systems can in this case be a compromise solution that allows us to combine the advantages of two types of amphiphiles, i.e., reduced toxicity due to the presence of nonionic surfactants and high efficiency due to the presence of cationic surfactants [15–17].

Continuing our series of studies of cationic surfactants that contain a cyclic head group [18–22], mixed micellar solutions of 1-methyl-1-hexadecyl-3-hydroxypiperidinium bromide (HPP-16) and Tween 80 were tested in this study. By varying the ratio of components in binary solutions, a number of aggregation characteristics were obtained and their solubilization effect was tested using two hydrophobic biologically active compounds, bioflavonoid quercetin and fungicide carboxin, as an example.

To clarify the role of hydroxyl fragments, the properties of 1-methyl-1-hexadecylpiperidinium bromide (PP-16), an unsubstituted piperidinium analog, were tested in parallel. The formulas of the studied compounds are shown below:



EXPERIMENTAL

Tween 80 and quercetin from Sigma-Aldrich and carboxin from OOO Avgust (Russia) with a purity of 99% were used in the study. The reactions of *N*-methyl-3-hydroxypiperidine and *N*-methylpiperidine with hexadecyl bromide in ethanol with subsequent recrystallization of the reaction mixture according to the published procedure [22] were used for the synthesis of cationic surfactants 1-methyl-1-hexadecyl-3-hydroxypiperidinium bromide (HPP-16) and 1-methyl-1-hexadecylpiperidinium bromide (PP-16), respectively.

The aggregation properties of piperidinium surfactants were studied tensiometrically on a K6 tensiometer from Krüss GmbH (Germany) in solutions thermostatted at 25°C. Under the conditions of an experiment with pH in the range of 4.4–7.0, HPP-16 was present in the studied solutions in cationic form.

The absorption spectra of the solutions were recorded in a Specord 250 Plus spectrophotometer using thermostatic quartz cells with an absorbing layer thickness of 1 cm. The molecular extinction coefficient (ϵ) of solubilizates in the studied solutions was calculated from the optical density (D) of the sample with a given solubilizate concentration (C) at the absorption maximum according to equation $\epsilon = D/(LC)$, where L is the thickness of the absorbing layer.

The surface potential of aggregates was estimated spectrally, studying the dependence of the acid–base properties of the indicator (*p*-nitrophenol) on the concentration of surfactants in accordance with [23, 24]. The observed pK_a value of *p*-nitrophenol ($pK_{a,\text{eff}}$) was calculated using the Henderson–Hasselbach equation

$$pK_{a,\text{eff}} = \text{pH} + \log \frac{[\text{phenol}]}{[\text{phenolate}]} \quad (1)$$

The value of the observed constant of dissociation when $C_{\text{surf}} \rightarrow \infty$ was taken as that of *p*-nitrophenol in the micellar phase ($K_{a,m}$).

The solubilizing effect micellar systems have on hydrophobic compounds was measured by determining the maximum achievable concentration of quercetin (or carboxin) in surfactant solutions in a way similar to the one described in [17, 24]. The ability of surfactants to increase the transport of carboxin into the plant was tested on potato tubers. To accomplish this, the tubers were kept for a day in a 0.5 mM carboxin solution without additives, or with the addition of a surfactant (0.2 wt %). Carboxin was then extracted from the treated potato into double-distilled water, and the content of the fungicide in the extract was determined spectrophotometrically.

RESULTS AND DISCUSSION

The critical micelle concentration (CMC) is a key characteristic of surfactant solutions that in most cases determines the extent of their functional activity. The CMC values were determined for HPP-16/Tween 80 binary systems on the basis of surface tension isotherms (Fig. 1). The obtained CMC values fell upon increasing the content of a nonionic surfactant in the system; however, the behavior of the CMC did not obey the law of ideal mixing. This can be seen by comparing the experimental and calculated CMC values determined using the Clint model [25]:

$$\frac{1}{C^*} = \frac{\alpha_1}{C_1} + \frac{\alpha_2}{C_2} \quad (2)$$

where α_1 and α_2 are the molar fractions of ionic and nonionic surfactants in the solution, respectively; and C^* , C_1 , and C_2 are the CMC values for the mixed system and its ionic and nonionic surfactants, respectively.

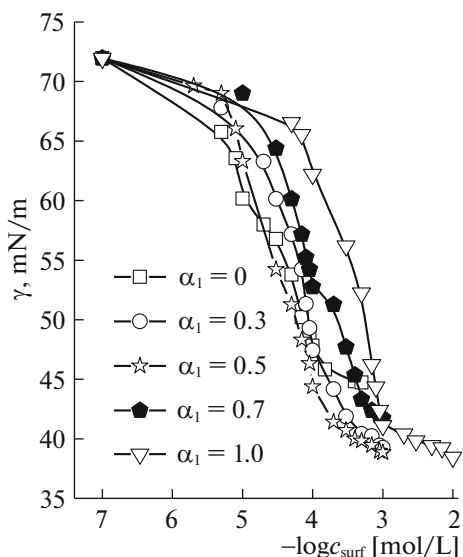


Fig. 1. Surface tension isotherms for the HPP-16/Tween 80 system for different ratios of surfactants at 25°C.

The negative deviation from the behavior of ideal mixing (Table 1 and Fig. 2) shows there is mutual attraction between different types of surfactants in micelles; i.e., a synergistic effect that is most pronounced for α_1 values in the range of 0.4 to 0.6.

Based on tensiometric data (Fig. 1), the adsorption parameters were calculated for the systems under study. The maximum adsorption value (Γ_{\max}) and the minimum surface area per surfactant molecule (A_{\min}) were estimated using the equations

$$\Gamma_{\max} = \frac{1}{2.3nRT} \lim_{C \rightarrow \text{CMC}} (d\pi/d \log C), \quad (3)$$

$$A_{\min} = \frac{10^{18}}{N_A \Gamma_{\max}}, \quad (4)$$

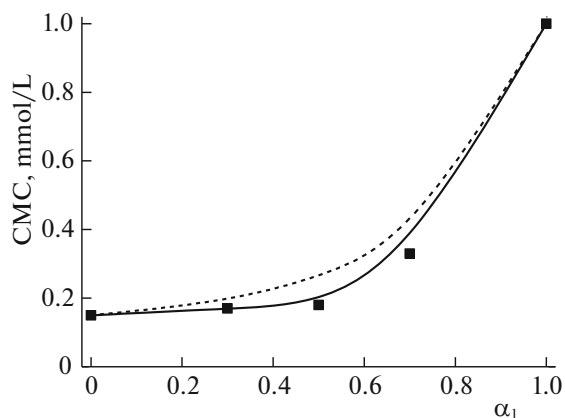


Fig. 2. Dependence of the CMC of solutions of surfactant mixtures on the molar fraction of HPP-16; the dashed line corresponds to an ideal system, and the symbols and continuous line correspond to the experimental dependence.

where $R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}$ (the universal gas constant); π is the surface pressure equal to the difference between the surface tension of the solvent and the solution at a given concentration of the surfactant (C); and T is absolute temperature (K). Parameter n takes a value of 2 for ionic surfactants comprised of a monovalent micelle-forming ion and a counterion. In Eq. (4), N_A denotes the Avogadro number ($6.02 \times 10^{23} \text{ mol}^{-1}$). The value of the free energy of micelle formation (ΔG_m) and the standard free energy of adsorption (ΔG_{ad}) were calculated with the equations

$$\Delta G_m = (1 + \beta)RT \ln(\text{CMC}), \quad (5)$$

$$\Delta G_{\text{ad}} = \Delta G_m - \frac{\pi_{\text{CMC}}}{\Gamma_{\max}}, \quad (6)$$

where β is the degree of counterion.

Our results are presented in Table 1. As for most surfactants, adsorption and micelle formation are thermodynamically advantageous processes for the

Table 1. Values of the CMC; maximum adsorption (Γ_{\max}); minimum surface per surfactant molecule (A_{\min}); free energy of micelle formation (ΔG_m); standard free energy of adsorption (ΔG_{ad}); and surface potential (Ψ) for mixed HPP-16/Tween 80 systems under conditions of varying the ratio of surfactants (α_1 is the fraction of cationic surfactant)

α_1	CMC, mmol		A_{\min} , nm ²	$\Gamma_{\max} \times 10^6$, mol m ⁻²	ΔG_m , kJ mol ⁻¹	ΔG_{ad} , kJ mol ⁻¹	pK _a	Ψ , mV
	tens.	calc.						
0	0.15		1.47	1.13	-33.3	-56.4	7.6	~0
0.3	0.16	0.19	1.19	1.40	-32.8	-53.9	7.0	35
0.5	0.18	0.26	1.21	1.38	-33.3	-55.4	6.5	65
0.7	0.33	0.37	1.25	1.33	-30.3	-52.1	6.0	94
1.0	1.0		0.89	1.87	-26.1	-43.1	5.7	112

The pK_a value for *p*-nitrophenol at $C_{\text{surf}} = 5 \text{ CMC}$, which is used to estimate Ψ .

Table 2. Solubilizing capacity of mixed micellar solutions with respect to quercetin (pH 4.4) and the extinction coefficients of the solubilize in the studied solutions at 374 nm

Fraction of ionic surfactant, α_1	ϵ_{374} , L mol ⁻¹ cm ⁻¹	S HPP- 16/Tween 80	S PP- 16/Tween 80
0	19000	0.040	0.040
0.3	19100	0.066	0.058
0.5	20500	0.153	0.137
0.7	19500	0.115	0.103
1	20000	0.240	0.180

studied compounds, both in the individual state and as a component of the mixed composition, and proceed spontaneously (the free energies of adsorption and micelle formation take negative values). These processes are facilitated in proportion to an increase in the fraction of a nonionic surfactant in the system, which is reflected in the growth of the absolute ΔG_m and ΔG_{ad} values. The values of maximum adsorption increase with an increase in the fraction of a cationic surfactant in the system, while the area occupied by a surfactant molecule in a saturated adsorption layer shrinks, which indicates denser packing of the molecules in the surface layer.

The formation of mixed systems lowers the charge of a micelle. To determine the surface potential (Ψ) of micelles, we studied the spectral properties of hydrophilic probe molecules capable of participating in acid–base equilibria [23]. The role of such a probe was played by *p*-nitrophenol, whose pK_a values were determined from spectra recorded by varying the ratio of components in an HPP-16/Tween 80 system in the pH range of 2 to 12, in a way similar to the one described in [24]. The shift of pK_a of this compound in solutions of surfactants compared to the one in water depends primarily on electrostatic interactions, the strength of which is determined by the surface potential of the micelle. The value of the surface potential was estimated with the formula

$$pK_{a,m} = pK_{a,0} - F\Psi/2.303RT, \quad (7)$$

where $pK_{a,0}$ is a nonelectrostatic component defined as $pK_{a,m}$ in micellar solutions based on nonionic surfactants; $F = 96485$ C mol⁻¹ is the Faraday constant; and $R = 8.314$ J K⁻¹ mol⁻¹ is the universal gas constant.

The Ψ values obtained in the mixed system are shown in Table 1. Upon an increase in the content of nonionic surfactant, a drop is observed in the surface potential, which is described by the linear equation

$$\Psi = 116\alpha_1 - 2.75 \quad (R = 0.989), \quad (8)$$

where α_1 is the fraction of a cationic surfactant. The change in the charge of a mixed micelle upon varying the ratio of components could be an important factor that determines its ability to bond guest compounds, its solubilizing capacity, the effect it has on the reactivity of solubilizes and their acid–base properties, and other characteristics.

In this work, we tested the solubilizing effect of mixed HPP-16/Tween 80 systems on quercetin. This biologically active substance with antioxidant and anti-inflammatory effects is nearly insoluble in water, but adding a surfactant can increase its concentration in solutions. The solubilizing effect micellar systems have on quercetin was determined by preparing a series of saturated solutions with it under conditions of varying the concentrations and ratio of the surfactants.

The concentration of quercetin in the test samples was controlled spectrophotometrically method. It was shown at the stage of preparation that the maximum absorption of quercetin in HPP-16/Tween 80 solutions at pH 4.4 remained virtually unchanged, regardless of the surfactant ratio, and was in the range of 374–376 nm; however, the coefficient of extinction grew slightly upon an increase in the fraction of a cationic surfactant (Table 2). For example, the absorption spectra of quercetin in HPP-16/Tween 80 solutions with a surfactant molar ratio of 1 : 1 are shown in Fig. 3. The solubilizing capacities (S) of individual and mixed micelles were calculated using the concentration dependences of the optical density (D) at the absorption maximum of the samples using the equation $S = b/\epsilon$, where b is the slope of dependence $D/L = f(C)$ at concentrations above the CMC; L is the cell thickness; C is the concentration of surfactant; and ϵ is the coefficient of extinction (Fig. 4). Our results are presented in Table 2. It follows from them that the solubilizing capacity of HPP-16 with respect to quercetin is much higher than the one with respect to Tween 80, reflecting the positive role of electrostatic interactions in the bonding of this solubilize to surfactant micelles. Mixed compositions are characterized by intermediate S values. A similar pattern was observed when using PP-16, an analog of HPP-16 that does not contain a hydroxyl group, to form mixed compositions (Table 2). The solubilizing capacity with respect to quercetin was in this case slightly lower than for systems based on HPP-16. This suggests that the efficiency of the solubilization of quercetin by micelles is determined not only by electrostatic and hydrophobic forces, but by specific interactions (hydrogen bonding) as well.

A somewhat different picture is observed for the solubilization of another studied hydrophobic compound, carboxin, which is a fungicide of systemic action. This biologically active substance belonging to the class of oxathiine derivatives is used in agriculture for the treatment of seeds and tubers. Like quercetin, it is poorly soluble in water. The procedure for deter-

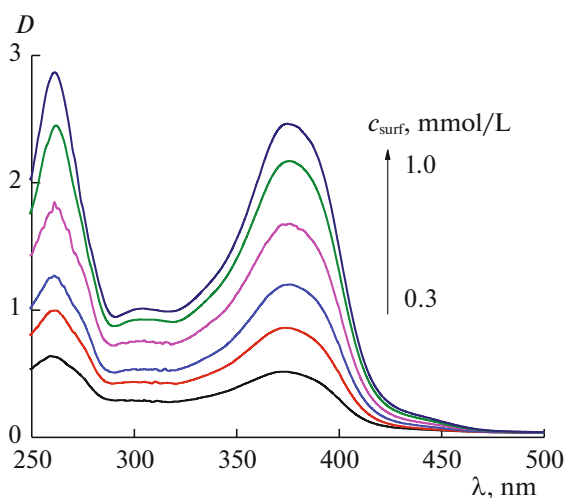


Fig. 3. (Color online) Absorption spectra of saturated solutions of quercetin as a function of the concentration of surfactants (HPP-16/Tween 80 system, $\alpha_1 = 0.5$; pH 4.4; 25°C).

mining the solubilizing capacity of mixed solutions was the same as described above. The optical density of solutions at the absorption maximum of carboxin at 297 nm, the position of which remained constant in all studied media, was taken as an analytical signal. The increase in the coefficient of molecular extinction that is observed upon an increase in the fraction of cationic surfactant, was considered when determining the maximum achievable concentration of carboxin in a solution.

The data for the HPP-16/Tween 80 systems with different ratios of surfactants are given in Table 3. They show that the presence of surfactants in a solution allows us to raise the content of carboxin in it, but no appreciable difference between the solubilizing effects of surfactants of different nature was found. This suggests that in this case, hydrophobic interactions play a dominant role. Since the practical use of fungicides in the treatment of seeds or tubers implies its effective adsorption on the treated surface and partial penetration into the plant material, and since cationic surfactants often exhibit a strong affinity for biosurfaces, we tested HPP-16 as an additive in carboxin solutions for the treatment of seed potato before planting. In accordance with agrochemical recommendations for the use of carboxin, potato tubers were kept for a day in a 0.5 mM aqueous solution of carboxin without adding surfactants, or with adding them at a concentration of 0.2 wt %. The substance was then extracted, and its content in the extract was determined spectrophotometrically. It was found that the content of carboxin in a tuber with a weight of 100 g was 3.2 mg after treatment using aqueous solutions with no added surfactants, and 5.9 and 8.3 mg after treatment with additions of Tween 80 and HPP-16, respectively.

Mixed systems are characterized by intermediate values. Obtained results can depend not only on the

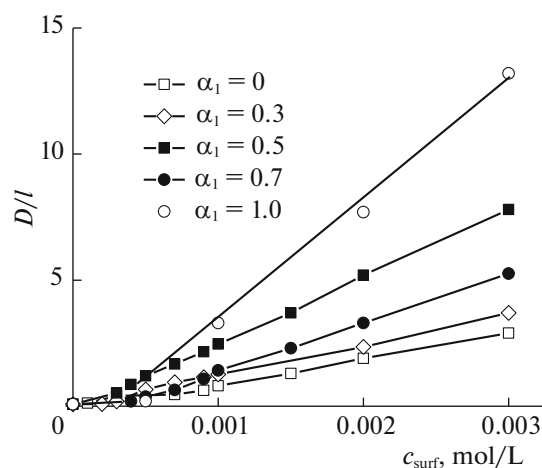


Fig. 4. Dependences of the optical density of HPP-16/Tween 80 solutions saturated with quercetin on the surfactant concentration ($\lambda = 375$ nm; pH 4.4; 25°C).

composition of the solution used in treatment, but on the properties of the tuber as well. It is therefore more correct to use relative parameters to estimate the transport properties of surfactants. It follows from this comparison that the efficiency of carboxin in solutions with surfactants, compared to aqueous solutions without surfactants, grows by a factor of 1.7 for Tween 80 and a factor of 2.5 for HPP-16. The latter cationic surfactant likely provides better adsorption of carboxin on the potato surface and to a greater extent facilitates the penetration of fungicide into the tuber.

CONCLUSIONS

The possibility of creating mixed compositions with reduced toxicity caused by the presence of non-ionic surfactants, and high efficiency resulting from

Table 3. Solubilizing capacity of HPP-16/Tween 80 mixed micellar solutions with respect to carboxin (pH 4.4)* and the extinction coefficients of the solubilize in the studied solutions at 297 nm

Fraction of ionic surfactant, α_1	b^{**}	ϵ_{297} , $\text{L mol}^{-1} \text{cm}^{-1}$	S
0	782	6800	0.115
0.3	594	6800	0.087
0.5	688	6800	0.101
0.7	525	6900	0.076
1	794	7300	0.109

* The solubility of carboxin in water is around 5.7×10^{-4} mol/L.

** b is the angular coefficient of dependence $D = f(C_{\text{surf}})$.

the presence of cationic surfactants characterized by strong solubilizing ability and transport properties with respect to hydrophobic substances, was demonstrated using the HPP-16/Tween 80 system as an example.

FUNDING

This work was supported by the Russian Foundation for Basic Research; and by the Government of the Republic of Tatarstan, project no. 18-43-160015.

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Translated by O. Kadkin