Determination of Critical Micellar Concentration of Homologous 2-Alkoxyphenylcarbamoyloxyethyl-Morpholinium Chlorides

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Abstract: The critical micellar concentrations of selected alkyloxy homologues of local anesthetic 4-(2-[(2-alkoxyphenyl)carbamoyl]oxy)ethyl)morpholin-4-ium chloride with \( n_c = 2, 4, 5, 6, 7, 8, \) and 9 carbons in alkyloxy tail were determined by absorption spectroscopy in the UV–vis spectral region with the use of a pyrene probe. Within the homologous series of the studied amphiphilic compounds, the \( \ln(cmc) \) was observed to be dependent linearly on the number of carbon atoms \( n_c \) in the hydrophobic tail: \( \ln(cmc) = 0.705–0.966 \, n_c \). The Gibbs free energy, necessary for the transfer of the methylene group of the alkoxy chain from the water phase into the inner part of the micelle at the temperature of 25 \( ^\circ\)C and \( pH \approx 4.5–5.0, \) was found to be \( -2.39 \, kJ/mol. \) The experimentally determined \( cmc \) values showed good correlations with the predicted values of the bulkiness of the alkoxy tail expressed as the molar volume of substituent R, as well as with the surface tension of the compounds.

Keywords: local anesthetic; morpholinium chloride; critical micellar concentration; pyrene absorption; sigmoidal Boltzmann equation

1. Introduction

Amphipathics are synthetic or natural molecules that covalently bond hydrophilic (soluble in water) and hydrophobic (insoluble in water) groups. In aqueous solution, such molecules self-assemble into aggregates of diverse forms and sizes [1]. These self-aggregated structures are habitually spherical assemblies [2–4], where the non-polar amphipathic tail groups are disposed within a hydrophilic shell provided by the polar head groups of the amphipathics, and the created species is called the micelle. Particularly spherical micelles can increase one-dimensionally into the cylindrical, or two-dimensionally into discoidal ones, or bilayers. This process depends on a solution and its conditions, as well as on the character of a surfactant. Surfactant’s polar groups ultimately control micelle extension. It happens because both one-dimensional growths cause that the polar groups to get closer to each other. This led to restriction of the available area of a surfactant molecule at the micelle surface and influenced curvature of the surface as well [5,6].

The surfactant molecules in aqueous solutions of these micelle structures are directed with their polar groups towards the aqueous phase, and with their hydrophobic fragments outside of this phase. Regarding ionic micelles, an interphase area between aqueous and micelle phases includes ionic polar groups, the Stern layer of the electrical double layer, which was merged with these groups, approximately a half of a counter ion connected with both aqueous environment and micelle. The leftover counter ions are surrounded within the Gouy–Chapman ratio of the double layer, which
goes further into the aqueous phase. In the case that the electrolytes are present, double layer length is a function of ionic power of the solution, and can be extremely compressed.

The Gibbs free increments per methylene group by different phase transitions $\Delta G^*(\text{CH}_2)$ can be provided as measuring the strength of the interactions between species in aqueous solutions of surfactant. For any homologous series of surfactants, the relationship between the critical micellar concentration $\text{cmc}$ and the number of carbon atoms $n_c$ in the hydrophobic group is defined as

$$\ln(\text{cmc}) = A - B n_c,$$

where $A$ and $B$ are constants reflecting the free energy changes caused in transferring the hydrophilic group and a methylene component of the hydrophobic group, respectively, from an aqueous surrounding to the micelle. The change in Gibbs free energy of transfer of any methylene group between the phases (system of two water-immiscible or partially water-miscible phases, respectively, for water ($w$) and micellar ($m$) phase at constant temperature ($T$), $\Delta G^*(\text{CH}_2)$, is given by [7–9]

$$\Delta G^*(\text{CH}_2) = -BRT,$$

where $R$ is the universal gas constant (8.3144598 JK$^{-1}$mol$^{-1}$) and $T$ is the absolute temperature.

There are various methods, like tensiometry, conductometry, fluorimetry, calorimetry, light scattering, and nuclear magnetic resonance (NMR) spectroscopy for determination of the critical micellar concentration ($\text{cmc}$) [10–15]. Spectral methods like absorption spectroscopy in ultraviolet/visible (UV–vis) region of spectrum, and fluorescence spectroscopy using other compounds as probes, are also used for the evaluation of $\text{cmc}$ [16,17].

In this article, we applied the pyrene absorption method [16] for determining the $\text{cmc}$ of the solution containing the series of selected morpholine derivatives of 2-alkoxyphenylcarbamic acid.

2. Results

These studied compounds (selected morpholine derivatives of 2-alkoxyphenylcarbamic acid) were evaluated on their local anesthetic potency [18]. It was found that their potency increases with the number ($n_c$) of carbon atoms in the alkoxy tail, up to hexyloxy and heptyloxy chain, respectively, and then decreases. The loss/evident decrease of biological activity at bigger chain lengths is familiar as a cut-off effect [19–21]. The effect of these compounds depends on membrane activity. The long alkyl chain that gets through the membrane and disrupts its structure is essential for the biological influence of this kind of compounds. It has been found that these characteristics can have an impact on biological efficacy of similar kinds of compounds [22–27].

Generally, surfactants are known to be membrane perturbants, with hydrocarbon tail integrated with the lipid bilayer of membranes [28,29]. This integration results in a disturbance of the membrane. The size of the tail of the surfactants is supposed to add to the extension of membrane damage, as longer chains can integrate into the lipid bilayers. The lowered activity of compounds with a longer tail (cut-off effect) may be accompanied by their limited solubility. Because the tail lengthens, lipid solubility rises at a quicker rate than that of partition coefficient change (lipid/aqueous). These longer tails are with limited partitioning, thus, the concentration at the place of activity is not adequate to have an important influence on the membrane of the cell wall [30]. However, the decrease of activity with the tail reaching a particular length may also be explained like this: the final methyl group of a compound can be found in the place of the final groups of the hydrocarbon chains of lipids that create the opposite monolayer, where surfactants are not integrated [20]. The presence of such “sewing” of both monolayers [14], and the terminal composition, can show a raised stability and lowered ability of biological action. This feature is usually named interdigitation, and has been studied for some amphiphilic compounds, alcohols, anesthetics, and other agents [31].

Surfactants are characterized by surface properties, therefore, molar volume (MV [cm$^3$]) and surface tension (ST [dyne/cm]) were predicted by means of ACD/Percepta (Advanced Chemistry
Development, Inc., Toronto, Canada). This software does not predict ionic compounds, therefore, both descriptors were calculated for 2-(morpholin-4-yl)ethyl (2-alkoxyphenyl)carbamates. The values are shown in Table 1.

In general, hydrophobic probes enter the micellar core and hydrophilic probes localize at the micellar surface. Thus, micellar structure may be examined by using a probe designed to localize in a particular micellar zone. This incorporation causes changes in intensities, respectively, in ratio of intensities of the peaks, in the characteristic absorption and emission spectra of the probe. The absorption spectrum of pyrene in water has showed eight peaks: strong (s) and weak (w) at 232w, 242s, 252w, 260w, 272s, 308w, 320s, and 336s nm, as depicted in Figure 1 [16,32,33]. The concentration of used pyrene was 2 µM, which was within its solubility limit of 2–3 µM. Excimer formation was expected to be absent at this concentration [16,34].

![Absorption spectrum of pyrene in water (2 µM) at 25 °C.](image1)

**Figure 1.** Absorption spectrum of pyrene in water (2 µM) at 25 °C.

In the absorption spectra of the studied compounds in water with pyrene concentration of 2 µM, the characteristic pyrene peaks at wavelengths of 336 and 320 nm were visible. In addition, the peak at 308 nm was also visible in derivatives 6 and 7. The other pyrene peaks were not observed due to the strong absorption of these surfactants in the near UV region, and thus, they were masked.

Plots of the sum of absorbances of all major pyrene peaks ($A_T$) against the surfactant concentration (c) have been found to be increasingly sigmoidal in nature (see Figure 2). The absorbances of each peak were also plotted separately against surfactant concentration in the inset.

![A_T vs. concentration (c) profile for compound 4 in aqueous medium at 25 °C (○); pH values vs. concentration (c) profile at 25 °C (○). Inset: Plots of absorbances (A) of individual pyrene peaks (at 336 and 320 nm) vs. surfactant concentration.](image2)

**Figure 2.** $A_T$ vs. concentration (c) profile for compound 4 in aqueous medium at 25 °C (○); pH values vs. concentration (c) profile at 25 °C (○). Inset: Plots of absorbances (A) of individual pyrene peaks (at 336 and 320 nm) vs. surfactant concentration.
Table 1. Structures of investigated 4-(2-[(2-alkoxyphenyl)carbamoyl]oxy)ethyl)morpholin-4-ium chloride 1–7, parameters of fitting functions with determined critical micellar concentration (cmc) values at 25 °C, and predicted values of molar volume (MV [cm³]) of substituent R and surface tension (ST [dyne/cm]).

| Comp. | R     | \(n_c\) | \(z\) | \(x_0\) | \(\Delta x\) | \(x_0/\Delta x\) | \(\chi^2\) | \(cmc_1\) [M] | \(cmc_2\) [M] | \(cmc\) [M] | MV * [cm³] | ST * [dyne/cm] |
|-------|-------|--------|------|--------|----------|------------|--------|----------|----------|----------|---------|---------------|
| 1     | C₂H₅  | 2      | 28   | 0.0760 | 2.17 × 10⁻² | 3.50       | 0.998  | 8.19 × 10⁻⁶ | 7.60 × 10⁻² | 1.19 × 10⁻¹  | 7.60 × 10⁻² | 47.29   | 46.40        |
| 2     | C₄H₉  | 4      | 42   | 0.0476 | 7.90 × 10⁻³ | 6.03       | 0.995  | 2.89 × 10⁻⁶ | 4.76 × 10⁻² | 6.34 × 10⁻²  | 4.76 × 10⁻² | 80.31   | 44.91        |
| 3     | C₅H₁₁ | 5      | 69   | 0.0397 | 2.76 × 10⁻² | 1.44       | 0.998  | 3.33 × 10⁻⁵ | 3.97 × 10⁻² | 9.49 × 10⁻²  | 3.97 × 10⁻² | 96.81   | 44.30        |
| 4     | C₆H₁₃ | 6      | 42   | 0.0172 | 6.29 × 10⁻³ | 2.73       | 0.998  | 2.63 × 10⁻⁵ | 1.72 × 10⁻² | 2.98 × 10⁻²  | 1.72 × 10⁻² | 113.32  | 43.76        |
| 5     | C₇H₁₅ | 7      | 28   | 0.0115 | 3.26 × 10⁻³ | 3.52       | 0.998  | 2.26 × 10⁻⁴ | 1.15 × 10⁻² | 1.80 × 10⁻²  | 1.15 × 10⁻² | 129.83  | 43.27        |
| 6     | C₈H₁₇ | 8      | 29   | 3.17 × 10⁻⁴ | 7.10 × 10⁻⁵ | 4.46       | 0.999  | 2.59 × 10⁻⁶ | 3.17 × 10⁻⁴ | 4.59 × 10⁻⁴  | 3.17 × 10⁻⁴ | 146.33  | 42.84        |
| 7     | C₉H₁₉ | 9      | 21   | 9.47 × 10⁻⁵ | 2.80 × 10⁻⁵ | 3.38       | 0.995  | 1.40 × 10⁻⁵ | 9.47 × 10⁻⁵ | 1.51 × 10⁻⁴  | 9.47 × 10⁻⁵ | 162.84  | 42.45        |

* Predicted using of ACD/Percepta v. 2012 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012) not for final hydrochlorides but for 2-(morpholin-4-yl)ethyl (2-alkoxyphenyl)carbamates.
Fitting the $A_T$ vs. concentration ($c$) profiles to the sigmoidal Boltzmann equation (SBE) was herein employed for cmc calculation [16,17,34–36]. Hence,

$$A_T = \frac{(a_i - a_f)}{1 + e^{(x-x_0)/\Delta x}} + a_f,$$

where $x$ is the overall concentration of surfactant, $a_i$ and $a_f$ are the upper and lower limits of the sigmoid, respectively, $x_0$ is the center of the sigmoid, and $\Delta x$ is directly related to the independent variable range where the abrupt change of the dependent variable occurs. The sigmoidal plot can produce two singular values of cmc, one at $x_0$ and the other at $(x_0 + 2\Delta x)$, respectively. Further, the ratio $x_0/\Delta x$ facilitates the decision which one is the right cmc [16,17]. The surfactant systems that provide $x_0/\Delta x < 10$ produce cmc1 = $x_0$, and those which yield $x_0/\Delta x > 10$ by the SBE process produce cmc2 = $(x_0 + 2\Delta x)$. For aqueous solutions of all studied derivatives, the ratio $x_0/\Delta x$ had the value of less than 10, indicating that the cmc is considered to be cmc1, and takes the value $x_0$ according to Aguiar’s theory [17]. The parameters of each sigmoidal function (the number of fitting points $z$, the number of carbon atoms in a hydrophobic tail $n_c$, $x_0$, $\Delta x$, the ratio $x_0/\Delta x$, the coefficient of determination $r^2$, chi-square $\chi^2$, value cmc1, cmc2, and cmc) are listed in the summary Table 1.

During the study of substances 5, 6, and 7, the significantly tarnished solutions occurred throughout the successive dilution process. At that moment, the absorbances significantly increased, and the solutions were not measurable until reaching the particular concentration at which a clear, and again, measurable solution was obtained; see Figures 3–5. Only in this low concentration range was there located the inflection point which determined the cmc (Figures 4 and 5).

![Figure 3. $A_T$ of main pyrene peaks at 336 and 320 nm vs. concentration ($c$) profile for compound 5 in aqueous medium at 25 °C in whole measured concentration range.](image)

One hypothesis for significant opalescence changes in solutions of compounds 5, 6, and 7 was the change in the pH value. Therefore, the further research has been moving in this direction. Dependence of the pH value on the concentration ($c$) of compounds 6 and 7 in aqueous solutions (Figures 4 and 5) showed that the pH was unlikely to have an effect on opalescence change, because (a) the pH value was almost constant before and during the tarnishing process, and (b) the same pH vs. concentration ($c$) profile was also seen for compound 4 (see Figure 2), where the opalescence change was not seen at all.

Another hypothesis for this process was that the phase transition and the change in micelle shape from a lower to higher level of complexity occurred at a certain concentration of surfactant in the solution. This hypothesis might be the basis for further examination to confirm this apparent change, for example, using the small angle neutron scattering (SANS) that could describe the structure of molecules and aggregates in solution [37,38].
In this study, the ln(cmc) values were calculated, and the plot of ln(cmc) vs. the number of carbon atoms, \( n_c \), in the hydrophobic chain is illustrated in Figure 6. The critical micellar concentration values decreases with increasing number of carbon atoms.

**Figure 4.** \( A_T \) of main pyrene peaks at 336 and 320 nm vs. concentration (c) profile for compound 6 in aqueous medium at 25 °C in whole measured concentration range (●); circle indicates concentration range where cmc was determined; pH values vs. concentration (c) profile at 2 °C (○).

**Figure 5.** \( A_T \) of main pyrene peaks at 336 and 320 nm vs. concentration (c) profile for compound 7 in aqueous medium at 25 °C in whole measured concentration range (●); circle indicates concentration range where cmc was determined; pH values vs. concentration (c) profile at 25 °C (○).

**Figure 6.** Dependence of ln(cmc) values on \( n_c \) in hydrophobic chain.
A study of micellization of the homologues of 2-alkoxyphenylcarbamoyloxyethylpyrrolidinium [39] and 2-alkoxyphenylcarbamoyloxyethylpiperidinium chloride [40] compared with 2-alkoxyphenylcarbamoyloxyethylmorpholinium chloride homologues in the distilled water medium and at temperature 25 °C showed that the CMC values increase in the order pyrrolidino-< morpholino-< piperidinoethyl derivatives. The most probable reason is the presence of the oxygen atom in the morpholine cycle of side chain, which remarkably changes the lipophilicity of the entire molecule in favor of its hydrophilic properties, which lead to increased solubility in water. Increased solubility in water can create a better environment for the formation of quantitative and qualitative parameters of micelles.

The ln(CMC) values of compounds with the number of carbon atoms \( n_c = 2, 4, 5, 6, 7, 8, \) and 9 are linearly dependent on \( n_c \): \( \ln(CMC) = 0.705 - 0.966 n_c \) with the coefficient of determination \( r^2 = 0.741 \). The CMC values decrease with increasing number of carbon atoms. However, it seems that the \( \ln(CMC) \) dependence on \( n_c \) deviates from linearity for substances \( n_c = 8 \) and 9, consequently, there is a low value for the coefficient of determination \( (r^2 = 0.741) \). In this case, the curve could probably have a quasi-parabolic dependence. This deviation is presumably due to longer alkoxy chain and the decreased solubility of these substances. They form micelles at low concentrations ranging from \( 10^{-5} - 10^{-4} \) M, as compared to homologues having a lower number of carbon atoms in the alkoxy chain, which form micelles at concentrations over \( 10^{-2} \) M. A large difference between these concentration values could probably cause the deviation from the linearity. The obtained experimental results support a model, according to which the quasi-parabolic dependencies of biological potencies of amphiphilic compounds on the length of their hydrophobic substituent (“cut-off” effect) could be caused by structural defects in lipid bilayer of the membrane induced by amphiphilic compounds or by micellization [19,40]. The Gibbs free energy \( \Delta G^\circ(CH_2) \), necessary for the transfer of the methylene group of the hydrophobic chain from an aqueous phase into the micelle interior at the temperature of 25 °C and pH ≈ 4.5–5.0, has, according to Equation (2), the value of \(-2.39 \) kJ/mol. For alkoxy homologues of local anesthetic heptacainium chloride determined by the ion-selective electrode, the value of \( \Delta G^\circ(CH_2) \) in water and in the 0.1 M NaCl solution is \(-1.21 \) kJ/mol and \(-1.54 \) kJ/mol, respectively [41]. It is assumed that the differences between these two results might be caused by the different species of the polar groups of two anesthetics, and by the composition of the system.

Using the Equations (1) and (2), and the experimental value of \( B = 0.966 \) given in Figure 6, the free energy change \( \Delta G^\circ(CH_2) \), involved in the transfer of the methylene group of the hydrophobic chain from an aqueous phase into the micelle internal, is negative, accordingly favoring micellization. This accounts also for the fact that the CMC values decrease with increasing length of the hydrophobic group. Using Equation (1) and the value of \( A = 0.705 \) depicted in Figure 6, the free energy change, involved in the transport of the hydrophilic group from an aqueous surroundings to the micelle external, is positive, and then goes up against micellization.

As mentioned above, the discussed compounds can be considered as surfactants, i.e., their biological effect is connected with the increasing surface activity (i.e., a longer tail as R substituent is preferable). This parameter is reflected in the bulkiness/molar volume (MV [cm\(^3\)]) of the alkoxy chain (substituent R). The surface tension (ST [dyne/cm]) of the compound is also connected with the length of the alkoxy tail. The calculated values of surface tension increase (i.e., surface activity decreases) with decreasing bulkiness, expressed as molar volume (reflecting a decrease in the length of the alkoxy tail). The dependence of the CMC values of the discussed compounds on the bulkiness/molar volume (MV [cm\(^3\)]) of the alkoxy chain is illustrated in Figure 7. It can be stated that the CMC values of the compounds linearly decrease \( (n_c = 7, r^2 = 0.960) \) with increasing bulkiness (the length of the tail). On the other hand, but logically, the CMC values linearly increase \( (n_c = 7, r^2 = 0.979) \) with increasing surface tension (i.e., with a decrease of surface activity), see Figure 8, where the dependence of the CMC values on the surface tension of the compounds is illustrated. These linear dependences showed in Figures 7 and 8 underline the accuracy of the determined CMC values of the discussed compounds and their usefulness for investigation of compounds of a similar type. Our results showed a close
relationship between the experimentally determined \( cmc \) and surface activity, respectively, biological activity of the compounds.

![Graph](image)

**Figure 7.** Dependence of \( cmc \) values on bulkiness alkoxy tail expressed as molar volume (MV [cm\(^3\)]) of substituent R.

![Graph](image)

**Figure 8.** Dependence of \( cmc \) values on surface tension (ST [dyne/cm]) of whole compound.

### 3. Materials and Methods

#### 3.1. Chemistry

The investigated compounds were prepared according to Scheme 1 by Cizmarik et al. \[18,42\]. Briefly, the starting material 2-aminophenol with acetonhydride yielded 2-acetaminophenol that, by condensation with appropriate alkylbromide, gave a series of seven alkoxyacetanilides, the cleavage of which by an aqueous solution of hydrochloric acid provided the corresponding alkoxyanilines. By reaction with phosgene in anhydrous toluene, the latter yielded alkoxyphenylisocyanates, that gave 2-(morpholin-4-yl)ethyl (2-alkoxyphenyl)carbamates by reaction with 2-morpholinoethan-1-ol. Hydrochloride salts 1–7 (see Scheme 1 and Table 1) of these carbamates were prepared using an equimolar amount of ethereal hydrogen chloride.
Finally, studied compounds 1–7 were recrystallized before investigations. All studied compounds are white crystalline substances. The structural identity and purity of each of compounds were verified using high-resolution mass spectrometry, and were measured using a high-performance liquid chromatograph Dionex UltiMate® 3000 (Thermo Scientific, Waltham, MA, USA) coupled with a LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Fourier Transform Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) with injection into HESI II in the positive or negative mode. Pyrene (≥99%, Sigma-Aldrich, Buchs, Switzerland). Experimental assays were performed at laboratory temperature 25 °C. The critical micellar concentration (cmc) values were established by methods [16,17].

Scheme 1. Synthesis of 4-(2-[(2-alkoxyphenyl)carbamoyl]oxy)ethyl)morpholin-4-ium chloride 1–7.

Reagents and conditions: (a) (CH₃CO)₂O, room temperature, 1 h; (b) Na, EtOH, alkylbromides, reflux, 3 h; (c) 18% HCl, reflux, 2 h; (d) COCl₂, toluene, reflux, 3 h; (e) toluene, reflux, 3 h; (f) HCl, Et₂O [10,11].

3.2. Preparation of Pyrene Stock Solution

All investigated compounds 1–7 were easily soluble in the water. A store solution of pyrene was prepared by adding a known weight of the compound in 20 wt % ethanol in water. The mixture was sonicated in order to prepare a clear solution. Its dilution provided the experimental 2 µM solution of pyrene, in which a concentration of ethanol was 0.25%. Such a minimal concentration of ethanol was considered unable to influence the self-aggregation processes and spectral performance of amphiphiles as well.

3.3. Absorbance Measurements

An absorbance study was performed by UV–vis spectrophotometer Spekol 1300 (Analytic Jena AG, Jena, Germany) using 10 mm path length quartz cuvette. The spectra were recorded in the 200–400 nm wavelength scope.

3.4. pH Measurements

pH values of studied local anesthetics were measured by inoLab Cond 7110 pH meter (Xylem Analytics Germany Sales GmbH & Co., KG, WTW, Weilheim, Germany).

4. Conclusions

Critical micellar concentrations within a homologous series of 4-(2-[(2-alkoxyphenyl)carbamoyl]oxy)ethyl)morpholin-4-ium chlorides were determined. These compounds were prepared as local anesthetics, i.e., surfactant-type compounds; therefore, their surface activity characterization is important for their biological potential. UV absorption of pyrene in surfactant solution has been an easy method to establish the cmc of surfactant. The values of cmc with the number of carbon atoms \( n_c = 2, 4, 5, 6, 7, 8 \), and 9 linearly dependent on \( n_c \): \( \ln(\text{cmc}) = 0.705 - 0.966 n_c \). The Gibbs free energy change \( \Delta G^* ) (\text{CH}_2) \), necessary for the transfer of the methylene group of the hydrophobic chain from an aqueous phase into the micelle interior, has a value of \(-2.39 \text{ kJ/mol} \) at 25 °C. It can be provided...
as a measure of the intensity of the interactions between species in aqueous solutions of surfactant. The experimentally determined \( \text{cmc} \) values showed good correlations with the predicted values of the bulkiness of the alkoxy tail, expressed as the molar volume (MV [cm\(^3\)]) of substituent R, as well as with the surface tension (ST [dyne/cm]) of the compounds.

**Author Contributions:** Lenka Stopková, Jana Gališinová, Zuzana Šuchtová and Fils Andriamainty conceived and designed the experiments; Lenka Stopková, Jana Gališinová and Zuzana Šuchtová performed the experiments using the UV–vis spectroscopy method; Lenka Stopková, Jana Gališinová and Fils Andriamainty analysed all measured and calculated data; Jozef Čizmárík contributed studied substances; Lenka Stopková and Fils Andriamainty wrote the paper; all authors equally contributed to final paper revising.

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**References**

1. Rong, L. *Water-Insoluble Drug Formulation*, 2nd ed.; CRC Press: London, UK, 2008; pp. 260–261.
2. Geng, Y.; Ahmed, F.; Bhasin, N.; Discher, D.E. Visualizing worm micelle dynamics and phase transitions of a charged diblock copolymer in water. *J. Phys. Chem. B* 2005, 109, 3772–3779. [CrossRef] [PubMed]
3. Karumbamkandathil, A.; Ghosh, S.; Anand, U.; Saha, P.; Mukherjee, M.; Mukherjee, S. Micelles of Benzethonium Chloride undergoes spherical to cylindrical shape transformation: An intrinsic fluorescence and calorimetric approach. *Chem. Phys. Lett.* 2014, 593, 115–121. [CrossRef]
4. Acharya, D.P.; Kunieda, H. Formation of viscoelastic wormlike micellar solutions in mixed nonionic surfactant systems. *J. Phys. Chem. B* 2003, 107, 10168–10175. [CrossRef]
5. Chevalier, Y.; Zemb, T. The structure of micelles and microemulsions. *Rep. Prog. Phys.* 1990, 53, 279–371. [CrossRef]
6. Puuvada, S.; Blankschtein, D. Molecular thermodynamic approach to predict micellization phase behavior and phase separation of micellar solutions. I. Application to nonionic surfactants. *J. Chem. Phys.* 1990, 92, 3710–3724. [CrossRef]
7. Rodriguez, O.; Madeira, P.P.; Macedo, E.A. Gibbs free energy of transfer of a methylene group in buffer + ionic liquid biphasic systems. *Ind. Eng. Chem. Res.* 2008, 47, 5165–5168. [CrossRef]
8. Silverio, S.C.; Rodriguez, O.; Teixeira, J.A.; Macedo, E.A. Gibbs free energy of transfer of a methylene group on \{UCON + (sodium or potassium) phosphate salts\} aqueous two-phase systems: Hydrophobicity effects. *J. Chem. Thermodyn.* 2010, 42, 1063–1069. [CrossRef]
9. Rosen, M.J. *Surfactants and Interfacial Phenomena*, 3rd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2004; pp. 159–160.
10. ZakhARova, L.Y.; Gaysin, N.K.; Gnezdilov, O.I.; Bashirov, F.I.; Kashapov, R.R.; Zhiltsova, E.P.; Pashirova, T.N.; Lukashenko, S.S. Micellization of alkylated 1.4-diazabicyclo[2.2.2]octane by nuclear magnetic resonance technique using pulsed gradient of static magnetic field. *J. Mol. Liq.* 2012, 167, 89–193. [CrossRef] [PubMed]
11. Nyuta, K.; Yoshimura, T.; Esumi, K. Surface tension and micellization properties of heterogemini surfactants containing quaternary ammonium salt and sulfobetaine moiety. *J. Colloid Interface Sci.* 2006, 301, 267–273. [CrossRef] [PubMed]
12. Sajid Ali, M.; Abdul Rub, M.; Khan, F.; Al-Lohedan, H.A.; Kabir-ud-Din. Interaction of amphiphilic drug amitriptyline hydrochloride with β-cyclodextrin as studied by conductometry, surface tensiometry and viscometry. *J. Mol. Liq.* 2012, 167, 115–118.
13. Bai, G.; Lopes, A.; Bastos, M. Thermodynamics of micellization of alkylimidazolium surfactants in aqueous solution. *J. Chem. Thermodyn.* 2008, 40, 1509–1516. [CrossRef]
14. Moulik, S.P.; Mitra, D. Amphiphile self-aggregation: An attempt to reconcile the agreement-disagreement between the enthalpies of micellization determined by the van’t Hoff and Calorimetry methods. *J. Colloid Interface Sci.* 2009, 337, 569–578. [CrossRef] [PubMed]
15. Sarac, B.; Bester-Rogac, M. Temperature and salt-induced micellization of dodecyltrimethylammonium chloride in aqueous solution: A thermodynamic study. *J Colloid Interface Sci.* 2009, 338, 216–221. [CrossRef] [PubMed]
16. Basu Ray, G.; Chakraborty, I.; Moulik, S.P. Pyrene absorption can be a convenient method for probing critical micellar concentration (cmc) and indexing micellar polarity. *J. Colloid Interface Sci.* 2006, 294, 248–254. [CrossRef] [PubMed]

17. Aguiar, J.; Carpena, P.; Molina-Bolivar, J.A.; Carnero Ruiz, C.J. On the determination of the critical micelle concentration by the pyrene 1:3 ratio method. *J. Colloid Interface Sci.* 2003, 258, 116–122. [CrossRef]

18. Cizmarik, J.; Borovansky, A.; Tumova, I. Untersuchungen an Lokalanästhetica. Teil 91: Morpholinoethylester der Alkoxyphephorylcarbamidsäuren. *Pharmazie* 1987, 42, 702–703. [PubMed]

19. Balgavy, P.; Devinsky, F. Cut-off effects in biological activities of surfactants. *Adv. Colloid Interface Sci.* 1996, 66, 23–63. [CrossRef]

20. Przestalski, S.; Sarapuk, J.; Kleszczynska, H.; Gabrielska, J.; Hladyszowski, J.; Trela, Z.; Kuczera, J. Influence of amphiphilic compounds on membranes. *Acta Biochim. Pol.* 2000, 47, 627–638. [PubMed]

21. Sarapuk, J.; Kublica, K. Cut-off phenomenon. *Cell. Mol. Biol. Lett.* 1998, 5, 261–269.

22. Good, N.E. Inhibitors of the Hill reaction. *Plant Physiol.* 1961, 36, 788–803. [CrossRef]

23. Imramovsky, A.; Pesko, M.; Monreal-Ferriz, J.; Kralova, K.; Vinsova, J.; Jampilek, J. Photosynthesis-inhibiting efficiency of 4-chloro-2-(chlorophenylcarbamoyl)phenyl alkyl-carbamates. *Bioorg. Med. Chem. Lett.* 2011, 21, 4564–4567. [CrossRef] [PubMed]

24. Zadrazilova, I.; Pospisilova, S.; Masarikova, M.; Imramovsky, A.; Monreal-Ferriz, J.; Vinsova, J.; Cizek, A.; Jampilek, J. Salicylanilide carbamates: Promising antibacterial agents with high in vitro activity against methicillin-resistant *Staphylococcus aureus*. *Eur. J. Pharm. Sci.* 2015, 77, 197–207. [CrossRef] [PubMed]

25. Gonec, T.; Pospisilova, S.; Holanova, L.; Stranik, J.; Cernikova, A.; Pudelkova, V.; Kos, J.; Oravec, M.; Kollar, P.; Cizek, A.; et al. Synthesis and antimicrobial evaluation of 1-[2-substituted phenyl]carbamoyl]naphthalen-2-yl carbanates. *Molecules* 2016, 21, 1189. [CrossRef] [PubMed]

26. Gonec, T.; Kralova, K.; Pesko, M.; Jampilek, J. Antimycobacterial *N*-Alkoxyphephorylhydroxynaphthalene carboxamides Affecting Photosystem II. *Bioorg. Med. Chem. Lett.* 2017, 27, 1881–1885. [CrossRef] [PubMed]

27. Gonec, T.; Stranik, J.; Pesko, M.; Kos, J.; Oravec, M.; Kralova, K.; Jampilek, J. Photosynthesis-Inhibiting Activity of 1-[2-Chlorophenyl]carbamoyl]- and 1-[2-Nitrophenyl]carbamoyl]naphthalen-2-yl Alkylcarbamates. *Molecules* 2017, 22, 1199. [CrossRef] [PubMed]

28. Jampilek, J.; Brychtova, K. Azene analogues: Classification, design, and transdermal penetration principles. *Med. Res. Rev.* 2012, 32, 907–947. [CrossRef] [PubMed]

29. Kralova, K.; Sersen, F. Effects of bioactive natural and synthetic compounds with different alkyl chain length on photosynthetic apparatus. In *Applied Photosynthesis*; Najafpour, M., Ed.; InTech: Rijeka, Croatia, 2012; pp. 165–190.

30. Birnir, C.R.; Malamud, D.; Schnaare, R.L. Antimicrobial evaluation of N-alkyl betaines and N-alkyl-N,N-dimethylamine oxides with variations in chain length. *Antimicrob. Agents Chemother.* 2000, 44, 2514–2517. [PubMed]

31. Lobbecke, L.; Cevc, G. Effects of short-chain alcohols on phase behaviour and interdigitation of phosphatidylcholine bilayer membranes. *Biochim. Biophys. Acta* 1995, 1237, 59–69. [CrossRef]

32. Khan, Z.H.; Khanna, B.N. Electronic absorption spectra of pyrene and its monopositive ion. *J. Chem. Phys.* 1973, 59, 3015. [CrossRef] [PubMed]

33. Kalyansundaram, K.; Thomas, J.K. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. *J. Am. Chem. Soc.* 1977, 99, 2039–2044. [CrossRef]

34. Galisinova, J.; Andriamainty, F.; Malik, I.; Cizmarik, J.; Sichrovska, L. Study of local anaesthetics. Part 201: Determination of the critical micellar concentration of pentacaine hydrochloride from the measurements of UV absorption of pyrene in methanol solutions. *Ceska Slov. Farm.* 2013, 62, 132–135. [PubMed]

35. Galisinova, J.; Andriamainty, F.; Malik, I.; Cizmarik, J.; Karlovska, J.; Sichrovska, L. Study of local anaesthetics. Part 202: Determination of the critical micellar concentration of carbisocainium chloride in water using spectral methods and the probe pyrene. *Acta Fac. Pharm. Univ. Comen.* 2013, 60, 1–6.

36. Galisinova, J.; Andriamainty, F.; Cizmarik, J.; Malik, I.; Sichrovska, L.; Balazova, A. Study of local anaesthetics. Part 203: Micellization of heptacainium chloride studied by spectral methods in aqueous solution. *J. Bioequiv. Bioavailab.* 2013, 5, 161–164. [CrossRef]
37. Chen, S.H. Small angle neutron scattering studies of the structure and interaction in micellar and microemulsion systems. *Annu. Rev. Phys. Chem.* **1986**, *37*, 351–399. [CrossRef]

38. Juskowiak, B. Nucleic acid-based fluorescent probes and their analytical potential. *Anal. Bioanal. Chem.* **2011**, *399*, 3157–3176. [CrossRef] [PubMed]

39. Cizmarik, J.; Andriamainty, F.; Malik, I.; Sedlarova, E. Study of local anaesthetics. Part 178: Study of micellization of pyrrolidinoethyl esters of 2-alkoxysubstituted phenylcarbamic acid. *Farm. Obzor.* **2007**, *76*, 263–267.

40. Andriamainty, F.; Cizmarik, J.; Uhrikova, D.; Balgavy, P. Study of local anesthetics. Part 168. Critical micelle concentration of alkylxy homologs of local anesthetic heptacainium chloride determined by ion sensitive electrode. *Sci. Pharm.* **2005**, *73*, 17–25.

41. Balgavy, P.; Andriamainty, F.; Yaradaikin, S.; Gallova, J.; Uhrikova, D. Molecular mechanism of the “cut-off” effect in biological potencies of amphiphilic compounds. *Scr. Med.* **1996**, *69* (Suppl. 1), 7–15.

42. Cizmarik, J.; Borovansky, A.; Svec, P. Study of local anesthetics. LII. Piperidinoethyl esters of alkoxyphenylcarbamic acids. *Acta Fac. Pharm. Univ. Comen.* **1976**, *29*, 53–80.

**Sample Availability**: Samples of compounds 1–7 are available from author F. Andriamainty and J. Čizmárik.