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Study of Local Anaesthetics. Part 203*. Micellization of Heptacainium Chloride Studied By Spectral Methods in Aqueous Solution

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Abstract

The formation of micelles of the local anaesthetic heptacainium chloride (substance, XIX) in aqueous solution was investigated by the pyrene absorption and the pyrene I_1/I_3 ratio methods, respectively by optical density (OD) method for surface curvature measurement. First, the absorption spectroscopy in UV/VIS region was based on studying changes in characteristic absorption spectrum of pyrene in presence of surfactant. The resultant plot of the sum of absorbances for the entire key pyrene maximum as a function of the total surfactant concentration showed, in the region of the critical micellar concentration, a usual sigmoidal increase. The fluorescence emission spectroscopy in UV/VIS region of spectrum by the probe pyrene, second procedure, was applied for determination of the *cmc* from the detections of the pyrene I_1/I_3 ratio as a function of the surfactant concentration. The pyrene ratio data were fitted by the Boltzmann-type sigmoid of declining progress. The third (OD) method used required microliters of surfactant solutions and standard microplate absorbance measurements.

Introduction

The process of self-association of surfactants into micelles, vesicles and membranes maintain very important function in many fields, extending from biological systems to technical applications.

Micelles, which can be spherical, cylindrical, or planar (discs or bilayers), are unstable units formed by noncovalent aggregation of individual surfactant monomers. Micelle form and bulk can be forced by changing the surfactant chemical structure and by varying solution circumstances such as temperature alike, ionic strength, surfactant composition (in case of mixed surfactant systems), overall surfactant concentration and pH. Especially depending on the solution conditions and on the surfactant nature, spherical micelles are able to grow one-dimensionally into cylindrical micelles or two-dimensionally into bilayers or discoidal micelles. Micelle enlargement is controlled mainly by the surfactant polar groups, since both one-dimensional growth involve bringing the surfactant polar groups closer to each other in order to diminish the obtainable area per surfactant molecule at the micelle surface, and hence the bend of the micelle surface [1,2].

In aqueous media these micellar structures, the surfactant molecules are directed with their polar groups towards the water phase and their hydrophobic parts away from it. In ionic micelles, the interphase area between the aqueous and the micelle phase involves the ionic polar groups, the Stearn Layer of the electrical double layer correlated to these groups, approximately half of the counter ion linked with the micelle and water. The remaining counter ions are enclosed in the Gouy-Chapman ratio of the double layer that reaches further into the aqueous stage. In the presence of electrolytes the length of the double layer is a function of ionic strength of the solution and it can be exceedingly compressed. The structure for nonionic surfactant having a polyethylene oxide (PEO) polar part, is basically identical, excluding the counter ions from the outer area, but rather coils of hydrated polyethylene oxide chains. The inner side of the micelle including hydrophobic parts presents a radius of approximate length of the totally extended hydrophobic chain [3]. Micelles´ other significant feature is, that the aqueous stage penetrates into the micelle beyond the hydrophilic polar parts, and some of the first methylene hydrophobic parts nearby to the polar group are considered in the hydration area. Therefore, we can segregate the interior area of the micelle in an outer centre penetrated by water and in an inner centre completely water-excluded [2].

There are several used techniques such as NMR, surface tension, conductometry, light scattering and calorimetry to determine the critical micelle concentration (*cmc*) [4-9] and bulk thermodynamic properties like aggregation number [10-12]. In addition, spectral methods like UV/VIS and fluorescence using other substances as probes are also used for the assessment of *cmc* [13,14].

In this paper, we have tried to compare the pyrene absorption method [13] and the fluorescence method [14] in evaluating the *cmc* of the solution containing surfactant heptacainium chloride with the optical density method [15] uses changes of the meniscus curvature in high density multi-well plates related with colloidal changes in solution.

Experimental Section

Materials and Methods

The surfactant of cationic local anaesthetic heptacainium chloride was synthesized by Čižmárik et al. [16]. Pyrene was purchased from Sigma-Aldrich, Switzerland. Laboratory temperature throughout the experiment was 22°C. The critical micellar concentrations were determined by methods [13-15].

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Preparation of pyrene solution

A store solution of pyrene was arranged by adding a recognized weight of the substance in 20 wt% ethanol in water. The mixture was sonicated in direction to bring a clear solution. The experimental 2 µmol/l solution of pyrene was arranged from it by dilution in which the concentration of ethanol was 0.25%. That diminutive concentration of the ethanol was considered incapable to affect the self-aggregation and spectral performance of amphiphiles.

Pyrene absorbance method

In UV/VIS spectrophotometer Spekol 1300 Analytic Jena AG (Germany) the absorbance measurements were done using 10 mm path length quartz cuvette. The spectra were inscribed in the 200-400 nm wavelength scope.

Fluorescence method

Fluorescence measurements were done in Fluoro-Max-4 (Jobin Yvon) spectrofluorometer. Fluorescence emission spectra of a surfactant solutions figure containing 1-2 µmol/l of pyrene were inscribed using an excitation wavelength of 335 nm, and the intensities I_1 and I_3 were determined at the wavelengths relating to the first and third vibronic bands situated near 373 and 383 nm, respectively. The ratio I_1/I_3 is the entitled pyrene 1:3 ratio. All fluorescence measurements were carried out at 22°C.

Optical density method

Optical density measurements were made using an Epoch Microplate Spectrophotometer. Sample volumes were 200 µl in 96-micro well plates.

Results and Discussion

Pyrene absorption spectra

The absorption spectrum of pyrene in water has evidenced eight [13] peaks strong (s) and weak (w) at 232^w, 242^s, 252^w, 260^w, 272^s, 308^w, 320^s and 336^s nm, respectively, as shown in Figure 1. The concentration of used pyrene was 2 µmol/l which was within its solubility limit of 2-3 µmol/l. Excimer formation was supposed to be absent [13,17] at this concentration.

In the absorption spectrum of heptacainium chloride the pyrene peaks at 232, 242, 252, 260, 272 and 308 nm were not detected due to the strong inclusion of this surfactant in near UV area and the peaks of pyrene in issue were masked. The schemes of the total of absorbances of all the main pyrene peaks A_T (at 320 and 336 nm in heptacainium chloride water solution) against surfactant concentration in water showed around the cmc a typical sigmoidal shape of the increase in nature (Figure 2).

Fitting these profiles to the Sigmoidal-Boltzmann Eq. (1) was herein used for cmc calculation.

Hence,

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

Where is the total concentration of surfactant, a_i and a_f are the initial and final sigmoid asymptotes, in the order, x_0 is the sigmoid centre and Δx is the independent variable x interval. There can be created two singular cmcs by the sigmoidal plot, one at x_0 and the second at $(x_0 + 2\Delta x)$. Additionally the ratio $(x_0/\Delta x)$ can be a leader to
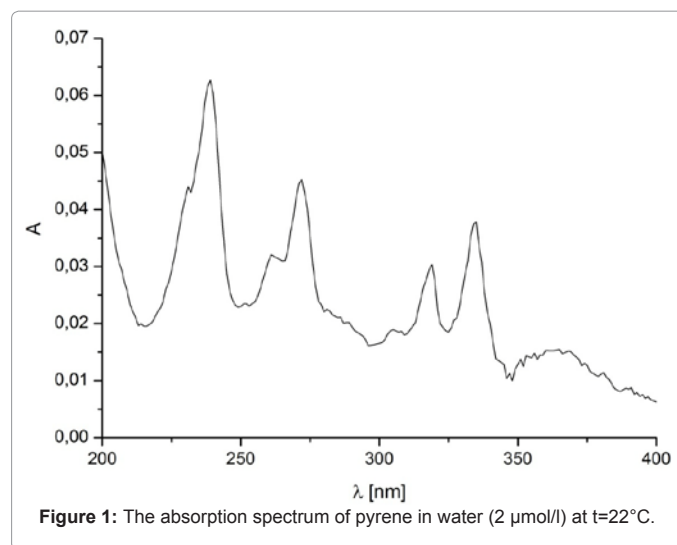

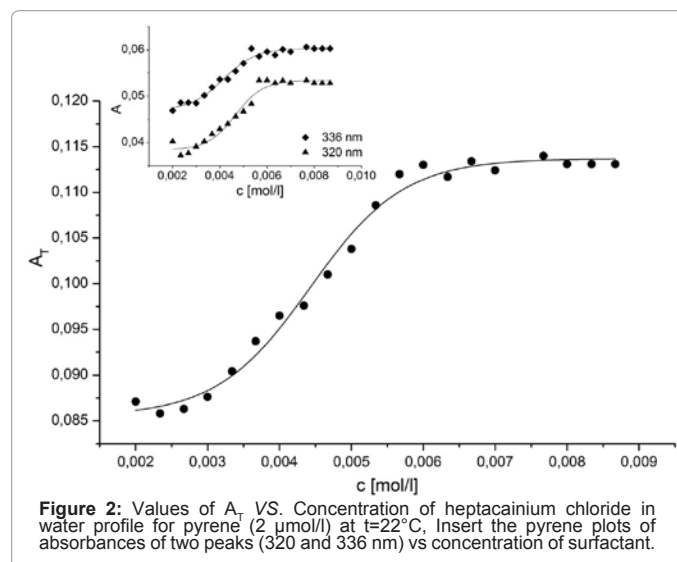**Figure 1:** The absorption spectrum of pyrene in water (2 µmol/l) at t=22°C.

Figure 2: Values of A_T *VS*. Concentration of heptacainium chloride in water profile for pyrene (2 µmol/l) at t=22°C, Insert the pyrene plots of absorbances of two peaks (320 and 336 nm) vs concentration of surfactant.

distinguish and to choose right cmc from the given possibilities. The surfactant structures that provide $(x_0/\Delta x) > 10$ by the (SBE) process produced cmc $2 = (x_0 + 2\Delta x)$.

The *cmc*1 and *cmc*2 values of heptacainium chloride and the most important fit parameters for surfactant schemes considered, counting the amount of aims used in the fit (n), regression- square (r^2) and the chi-sqaure (x^2) are presented in table. The absorption data treated by above mentioned equation(1) and analyzed in compliance with the suggestion of Aguair et al. [14] have formed $x_0/\Delta x$ value much lesser than 10, so that the cmc value was taken identical to x_0 as considered above [13,14].

Pyrene fluorescence spectra

In the emission spectra five emission summits at 373, 379, 383, 389 and 393 nm for pyrene (Figure 3) were observed. The so-called pyrene polarity index, I_1/I_3 (share of the first and third peaks intensities in the fluorescence emission range of pyrene) has turned out to be one of the most accepted procedures for the *cmc* determination in micellar systems [13,14,18]. Firstly we considered the pyrene monomer spectrum. The initial I_1/I_3 ratio decreased with the increase in surfactant

Method	n	x_0	Δx	$x_0/\Delta x$	r^2	χ^2	$cmc1$ (mol/l)	$cmc2$ (mol/l)
Absorption spectroscopy	20	0.00443	6.47×10^{-4}	6.84	0.989	1.34×10^{-6}	4.43×10^{-3}	5.72×10^{-3}
Fluorescence spectroscopy	28	0.00467	8.76×10^{-4}	5.33	0.994	8.36×10^{-5}	4.67×10^{-3}	6.42×10^{-3}
Optical density	16	0,00506	1.05×10^{-3}	4.82	0.983	1.57×10^{-5}	5.06×10^{-3}	7.16×10^{-3}

Table 1: The fit parameters, the quantity of points applied in the fit (n), regression -sqaure (r^2), the chi-sqaure (x^2) and the $cmc1$ and $cmc2$ values of heptacainium chloride.

concentration due to a increasingly less polar microenvironment [19]. A reduce in I_1/I_3 was observed reflecting the incorporation of the probe into micellar, non-polar environment [20]. This represents that the restricted polarity around the pyrene starts to decrease radically before micelles are observed [19].

Thus, the pyrene I_1/I_3 ratio plots could be sufficiently depicted by declining sigmoid of the

Boltzmann type (Figure 4), which was specified by Eq. (2):

$$Y = A_T = \frac{(a_i\_a_f)}{1 + e^{(x-x_0)/\Delta x}} + a_f \qquad (2)$$

Where the y corresponded to the pyrene I1/I3 ratio significance, the autonomous variable x was the entire surfactant concentration, a and a were the upper and lower restrictions of the sigmoid, correspondingly x was the centre of sigmoid, and Δx was in a straight line connected to the autonomous inconsistent scope where the sudden change of the reliant variable occurred.

Two singular *cmcs* can be created by the sigmoidal plot, the first at x_0 and the second at (x_0+2rx). The coefficient can be constructive to set up an objective approach when picking a particular point able to supply a consistent *cmc* value in the pyrene I_1/I_3 ratio plots.

In case of heptacainium chloride mentioned ratio was lower than 10 so that the cmc value was considered identical to chi-square ($\chi2$), and the (Basu Ray et al. [13] and Aguiar et al. [14]). In table 1 there were summarized the fit parameters, the quality of points applied in the fit (n), regression square (r^2), the chi-sqaure (x^2) and the *cmc1* and *cmc2* values of heptacainium chloride.
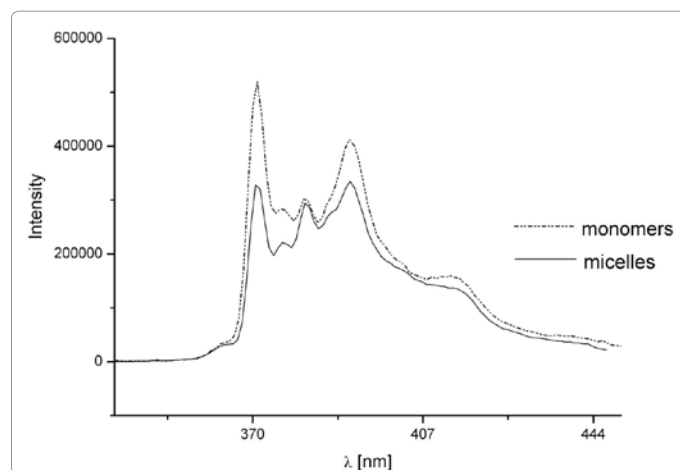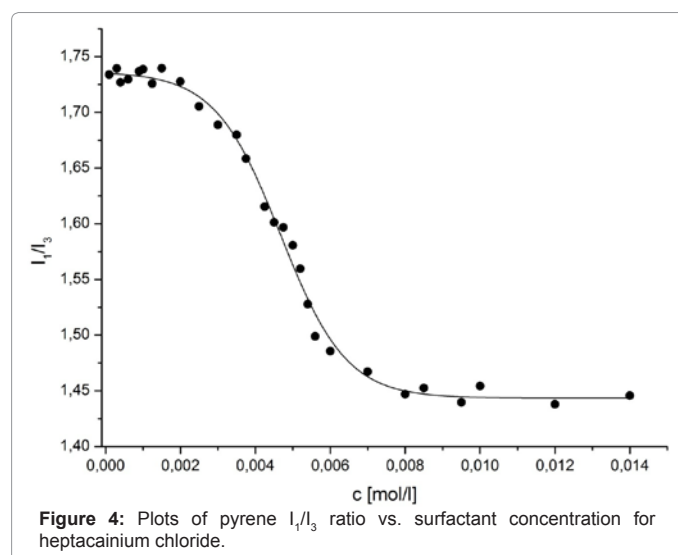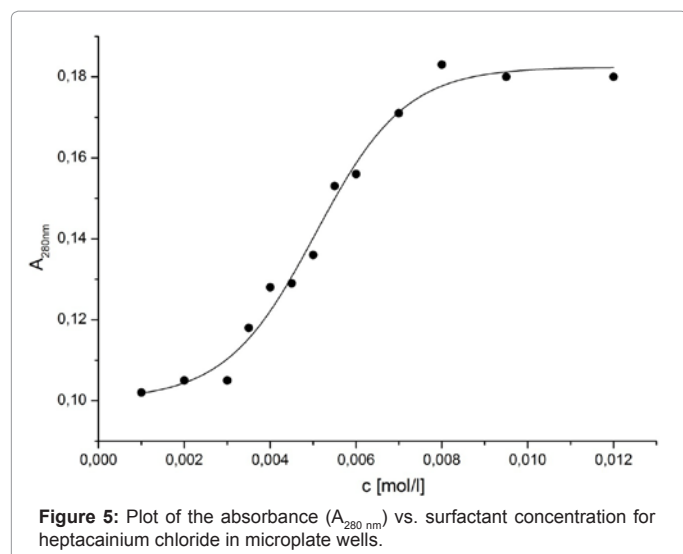

Figure 4: Plots of pyrene I_1/I_3 ratio vs. surfactant concentration for heptacainium chloride.

Surface curvature measurement by optical density method

The samples for cmc measurements were prepared by serial dilution of surfactant using a 96-well plate. The optical density method is reposed on the influence of a curved meniscus on spectrophotometric determinants applying a plate reader with a vertical light path, where the light path does not pass through an air-water interface and is horizontal. This method is founded on the Young equation (Eq. (3))

$$\gamma_{lv} \cos\theta = \gamma_{sv} - \gamma_{sl} \qquad (3)$$

Where, the contact angle θ is at the solid-liquid-vapour contact line to the surface tension of the liquid-vapour, and solid-vapour and the solid-liquid interfacial tensions respectively. The value of θ determines the meniscus curvature. When θ=90º, the meniscus is plain; when θ<90º, the meniscus is curved upwards at the ridges of the cuvette; when θ>90º, the meniscus is curved downwards. When optical density determinants are completed in 96-well plates including samples with different surface tensions, the lensing effect of the meniscus increases the quantity of light capable to get to the detector: the lower the value of θ<90º, the greater the curvature of the meniscus and the lower the intensity of the light detected. In the absorbance mode, an increased value for the optical density reflects in the meniscus-induced decline in intensity. Absorbance determinants are generally made with the central position of the beam of the well; in this arrangement, the maximal influence of a meniscus upon the absorbance read-out is 0.1-0.2 absorbance-surely sufficient to affect interpretation of results [15]. The absorbance vs. concentration curves are shown in Figure 5. Based on the mechanism of micelle formation, there was an inflection point in the surface tension vs. concentration curve at the surfactant cmc, which was observed in substance (XIX) tested.

Figure 3: The fluorescence spectrum of pyrene in water (2 µmol/l) at t=22°C.

Figure 5: Plot of the absorbance ($A_{280 nm}$) vs. surfactant concentration for heptacainium chloride in microplate wells.

Conclusion

This investigation has authorized us to reveal that the plots the total of the absorbances of all the main pyrene peaks vs. surfactant concentration could be adequately described by increasing Boltzmann-type sigmoid. This absorption profile enabled to define two singular points. The coefficient $x_0/\Delta x$ could be used to decide between one and the other value.

The typical emission spectrum of pyrene was characterized by decreasing sigmoid. The pyrene I_1/I_3 ratio resultant plot, as a surfactant concentration purpose, was used to determine the critical micellar concentration of heptacainium chloride. The *cmc* values of local anaesthetic heptacainium chloride obtained by pyrene absorption and fluorescence data were in good agreement with an optical density method.

Acknowledgment

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