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Effect of micelles on pK_a^* of acridine: a spectroscopic study

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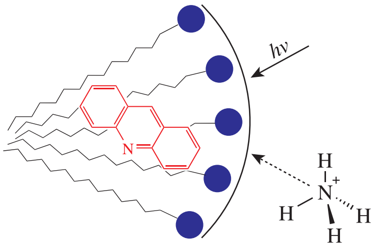
The effect of anionic, nonionic and cationic surfactants on the protonation reaction of acridine in an excited state has been studied. In the case of sodium decyl sulfate and sodium dodecyl sulfate, the observed shift in pK_a^* to a more alkaline region was +0.6 and +0.9 units (from 9.9 to 10.5 and 10.8, respectively), which corresponds to a decrease in the Gibbs free energy by 3.5 and 5.0 kJ mol^{-1}.



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Proton transfer remains one of the most fundamental processes in chemistry and biology.[1] In addition to the well-known reactions in the ground state, particular substances can enter into proton exchange reactions with a solvent or additives during the lifetime of an electronically excited state. Some compounds in an excited state become stronger acids, for example, hydroxy- and amino-substituted aromatic compounds; others, such as acridine or aromatic aldehydes, become stronger bases.[2–5]

Acridine and its derivatives have long been of interest to researchers for their ability to bind DNA and act as a good fluorophore.[6–11] They have found applications in microscopy, endomicroscopy, intraoperative fluorescence guidance, photodynamic therapy, sonodynamic therapy and radiodynamic therapy. A new line of research on acridines is aimed at their use in cancer theranostics due to their unique feature – predominant accumulation in the acidic environment of tumor tissues – and strong fluorescent properties. Based on the unique spectral differences and differential staining of RNA and DNA molecules, these substances could be used to distinguish between the stages of apoptosis and necroptosis. The protonation of Acridine Orange in micelles of the dye–surfactant ion pair was also investigated.[12] The use of acridine in a fluorescent pH sensor with a surfactant-loaded mesoporous silica substrate has been demonstrated.[13] It has been shown that noncovalent host–guest interactions can significantly shift the equilibrium constant pK_a (from 5.4 to 8.8, Δp$K_a \sim +3.4$), which could find potential applications in fluorescence off–on sensing and drug delivery.[14,15] Similar shifts are observed in the reactions of other acid–base indicators[16] and the photoinduced dissociation of naphthol derivatives in a polyelectrolyte field.[17,18]

Nevertheless, all the above-mentioned works on the protonation of acridine refer to the reaction in the ground state. This work is devoted to studying the influence of various micellar systems on the reaction in an excited state. Many real objects of analysis, especially biological ones, such as milk or blood components, often contain surface-active agents,[19] that can significantly affect the behavior of the analytical system.[20,21] For example, the possibility of interaction of Acridine Orange, both protonated and deprotonated forms, with aggregates of anionic surfactants in reverse micelles was shown earlier, which changed the photophysics of proton transfer.[22–24] Optical sensors represent an acceptable alternative to electrochemical methods,[25,26] and the study of organized media discussed in this work can expand the field of application.[27]

When acridine is excited to the S_1 state, referred to as Ac*, it becomes a much stronger proton acceptor. The pK_a of the acridinium cation AcH$^+$ increases from p$K_a = 5.45$ to p$K_a^* = 9.9$. These processes are depicted in Scheme 1, which illustrates all relevant photophysics.[28] The equilibrium constant can be written as

$$K_b = a_{AcH^+}\, a_{OH^-} / a_{Ac}. \tag{1}$$

For the ionization of water, the equilibrium constant is expressed as

$$K_W = a_{H^+}\, a_{OH^-}. \tag{2}$$

The expression of equilibrium constant for the reaction of acridine with H$^+$,

$$Ac + H^+ = AcH^+, \tag{3}$$

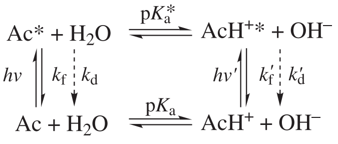
was obtained by dividing equation (1) by equation (2):

$$K_a = K_b / K_W = a_{AcH^+} / a_{Ac}\, a_{H^+}. \tag{4}$$

Thus, K_a is a relative property linking the basicity of acridine with the basicity of water. The magnitude of the change in the Gibbs free energy can be estimated using the well-known equation of the isotherm of a chemical reaction:[29]

$$\Delta G^0 = -RT \ln K_a. \tag{5}$$

In contrast to the deprotonation of a photoacid, a proton source is required for a noticeable protonation of a photobase in an alkaline



Scheme 1

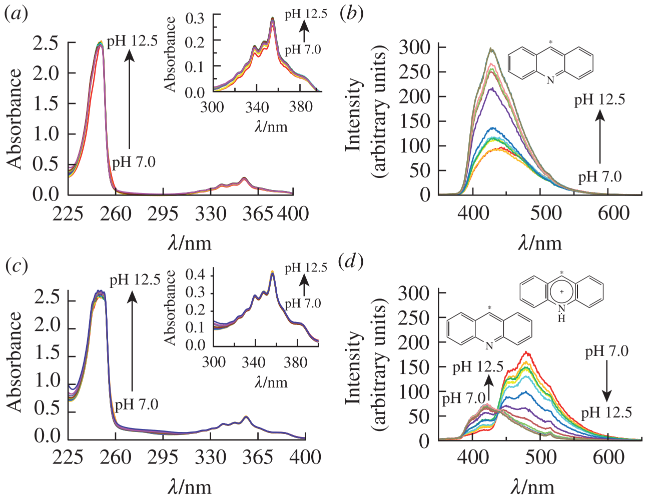


Figure 1 (*a*),(*c*) Absorbance and (*b*),(*d*) fluorescence spectra of acridine (6×10^{-5} M) in ammonium buffer solutions (0.2 M) in (*a*),(*b*) the absence and (*c*),(*d*) the presence of SDS (1.5 CMC).

medium. For this reason, all measurements were carried out in ammonium buffer solutions.[30]

The effect on the protonation reaction of acridine in the excited state was investigated for the following anionic [sodium octyl sulfate, sodium decyl sulfate (SDS) and sodium dodecyl sulfate (SDDS)], non-ionic (Triton X-100, Triton X-305 and Triton X-405) and cationic [decyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide and cetyltrimethylammonium bromide] surfactants. The experiments were carried out in buffer solutions with pH from 7.0 to 12.5 at a temperature of 25 ± 2 °C.[†]

The pH dependencies of absorbance and fluorescence spectra for acridine in the absence and the presence of an anionic surfactant are shown in Figure 1. It has recently been found that an Acridine Orange molecule with a similar structure can form dimers in micellar solutions.[31] Figure 1 demonstrates that the acridine-based system does not reveal signs of dimerization in the considered pH range from 7.0 to 12.5, as evidenced by the absence of changes in the structure of both absorption and emission spectra. Protonation of acridine occurs to a negligible extent at the used concentration of ammonium buffer, and the peak of the initial form Ac* predominates in the absence of surfactant [Figure 1(*b*)]. This observation is consistent with published results,[30] which indicate that the buffer concentration must be an order of magnitude higher for the complete protonation of Ac*. Nevertheless, the addition of an anionic surfactant dramatically alters the appearance of the spectrum under the same conditions [Figure 1(*d*)]. The emission peak corresponding to the protonated form AcH^{+*} becomes predominant. Changing the pH of the medium leads to a change in the peak intensity ratio of both acridine forms. It is important to note that the Ac* peak maximum is shifted to the high-frequency region, from 430 to 420 nm, which can be explained by a change in the local environment.

Processing the dependencies of both spectra gives S-shaped curves (Figure 2). The inflection point corresponds to the equilibrium constant pK_a^*. It can be seen that the addition of an anionic surfactant leads to a noticeable shift in pK_a^* towards higher pH values. Similar dependencies are observed for reactions in the ground state in systems with noncovalent host–guest interactions.[14] The main reason is the solubilization of the nonpolar acridine molecule in the hydrocarbon phase, which leads to a shift in the equilibrium of the reversible reaction. Apparently, in the case of anionic surfactants, the local concentration increases also due to the interaction of negatively charged micelles and ammonium cations.

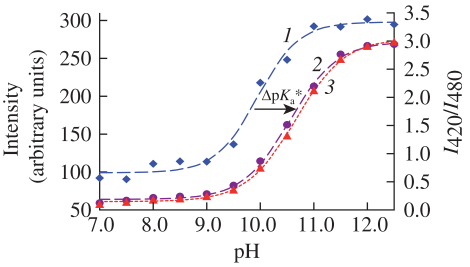


Figure 2 (*1*) Change in the fluorescence intensity of acridine at 430 nm depending on pH in the absence of a surfactant (left axis) and the pH-dependent ratio of the peak intensities of the initial Ac* and protonated AcH^{+*} forms of acridine (right axis) in the presence of 1.5 CMC of (*2*) SDS and (*3*) SDDS.

The latter conclusion is confirmed by comparing the effect of different types of surfactants on the experimental pK_a^* values (Figure 3). The introduction of nonionic surfactants into the system does not lead to a significant shift in the constant. The spectrum shape also does not change and corresponds to that observed for a solution of pure acridine. Charged surfactants with a short hydrocarbon chain also have no discernible effect. However, starting with SDS and DTAB, a significant shift in pK_a^* is observed. For systems with negatively charged surfactants, a shift towards higher pH values is noted, while in the case of cationic surfactants, on the contrary, a shift towards a more acidic region occurs. The latter can be explained by the Coulomb repulsion of positively charged micelles and ammonium cations.

The fluorescence quantum yields of acridine were determined for all series. The quantum yield naturally increases from 0.16 to 0.37 when the pH changes from 7.0 to 12.5 without surfactants. The dependence is similar to that shown in Figure 2, and the inflection point coincides with the pK_a^* value. The same S-shaped relationships are observed when adding surfactants to the system, with the difference that the range of change is from 0.03 to 0.10 with increasing pH. Such a significant drop in the quantum yield confirms the presence of the indicator mainly in the nonpolar micelle core.[32,33]

Thus, this work clearly shows that, even though acridine is predominantly in the micellar phase,[13] the Coulomb attraction of an anionic surfactant can create a local concentration of ammonium ions sufficient for a significant shift in pK_a^*. It is important to note that the proton transfer process across the micellar interface occurs during the lifetime of the excited state, which for the deprotonated and protonated forms of acridine is 10 and 31 ns, respectively.[13] The decrease in the Gibbs free energy when an

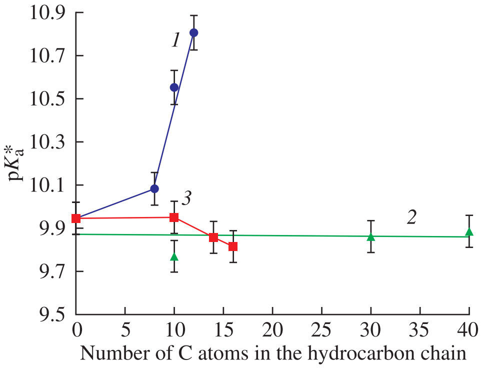


Figure 3 Shifts in the pK_a^* constant depending on the type of surfactant and the length of the hydrocarbon chain for (*1*) anionic, (*2*) nonionic and (*3*) cationic surfactants.

[†] For details, see Online Supplementary Materials.

anionic surfactant is introduced into the system can be estimated using equation (5). It is 3.5 and 5.0 kJ mol^{-1} for SDS and SDDS, respectively.

The observed effect upon the addition of surfactants is fundamentally different from the effect of polyelectrolytes, which do not affect the photoinduced proton transfer reaction but significantly shift the equilibrium of the acid–base reaction in the ground state.[17,18] Supramolecular tuning of pK_a and pK_a^* is of immense interest today as such systems find application in drug delivery, catalysis and sensor applications.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.021.

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