Halofantrine Incorporates in Sodium Dodecyl Sulfate (SDS) Micelles: Application in Fluorescence and Capillary Electrophoresis Studies

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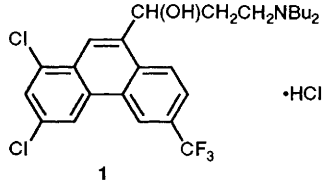
Fluorescence studies in H_2O and D_2O solutions have shown that halofantrine binds strongly to SDS micelles; this property makes this molecule an excellent marker to determine the migration time of micelles in micellar electrokinetic capillary chromatography (MECC).

The hydrochloride of 1-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3-*N*,*N*-dibutylaminopropan-1-ol **1**, generally known as halofantrine, is currently being marketed by SmithKline Beecham as a very effective wide spectrum anti-malarial drug.[1,2] This compound, dissolved in water, shows intense absorbance of ultra-violet radiation in the wavelength range of 200 to 300 nm (λ 220 nm, ε 3.2×10^4; 240 nm, ε 3.9×10^4). Its fluorescence is sufficiently intense to allow detection[3] well below 5 femtomoles. The solubility of **1** in water increases appreciably in the presence of sodium dodecyl sulfate (SDS), indicating inclusion in the micelle probably by both hydrophobic and electrostatic binding (at a pH below about 9.5).

The above spectroscopic and solubility properties of halofantrine, coupled with our interests in capillary electrophoresis (CE) and in the use of fluorescence probes to understand the solubilisation of molecules in micelles, compelled us to study in some detail the inclusion of **1** in SDS. The variations in the structure of SDS in solutions of H_2O and D_2O have been



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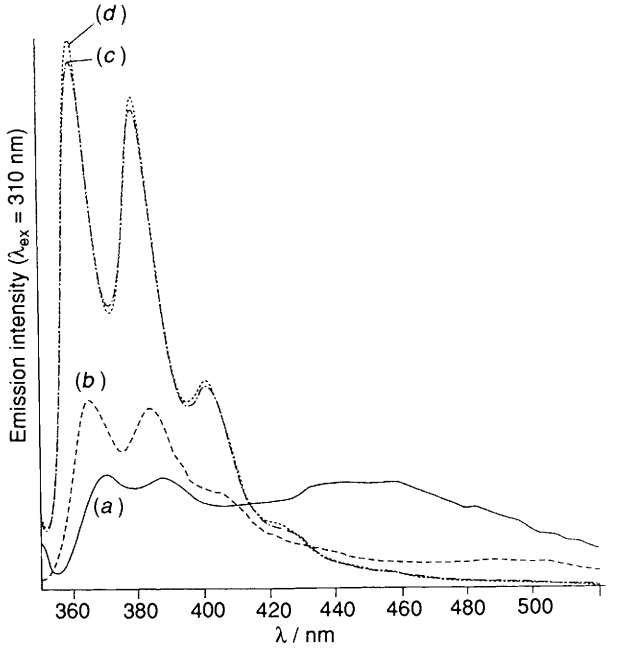

Fig. 1 Emission spectra of a 2.0 μmol dm^{-3} solution of halofantrine in (*a*) buffer, pH 7.03; (*b*) buffer, pD 7.02; (*c*) 8 mmol dm^{-3} SDS in buffer, pD 7.02

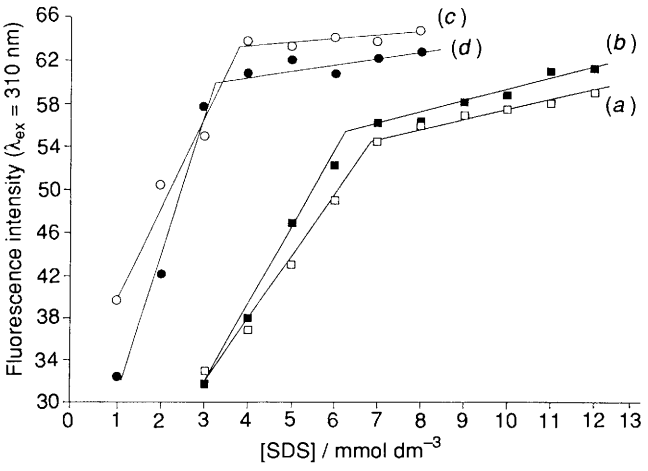

Fig. 2 Variation of fluorescence intensity of halofantrine with SDS concentration: (*a*) □ water; (*b*) ■ D_2O; (*c*) ○ phosphate–borate buffer (pH 7.01) and (*d*) ● phosphate–borate buffer (pD 7.01). The temperature of the cell compartment was 27 °C.

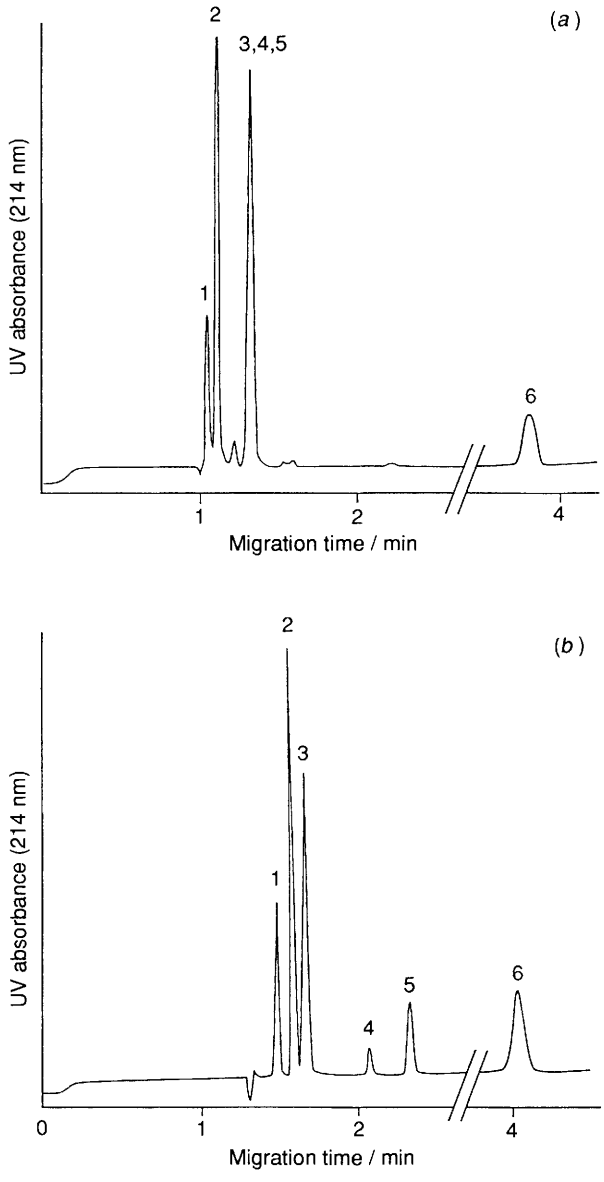

Fig. 3 Electropherograms of phenols (1) to (5) using SDS micelles in (*a*) H_2O-based buffer and (*b*) D_2O-based buffer solution. Peak (6) denotes the migration time of halofantrine. Length of capillary, 27 cm; radius of capillary, 50 μm; voltage, 10 kV; temperature outside capillary 27 °C; buffer: sodium phosphate (30 mol dm^{-3}) and boric acid (10 mmol dm^{-3}) adjusted to pH or pD 7.01 with NaOH or NaOD.

extensively detailed in the literature.[4–6] We now report fluorescence and micellar electrokinetic capillary chromatography (MECC) measurements of the inclusion of halofantrine **1** in SDS using both H_2O and D_2O based buffer solutions.

Emission spectra (excitation wavelength, 310 nm) of **1** in buffer (30 mmol dm^{-3} NaH_2PO_4 and 10 mmol dm^{-3} H_3BO_3 adjusted to the required pH or pD with NaOH or NaOD) at a pH or pD of 7.02 and in the presence of SDS are shown in Fig. 1. The concentration of **1** in all cases was 2.0 μmol dm^{-3}. Solutions were allowed to stand for about 3 h and were not deoxygenated prior to measurement. Fluorescence measurements were carried out at 27 °C on a Perkin Elmer LS50 spectrofluorimeter. The fluorescence intensity of **1** in H_2O-based buffer is remarkably different from that in D_2O under the same conditions [compare Fig. 1(*a*) to (*b*)]. The shift in H_2O to longer wavelengths and the appearance of a new wide peak at around 450 nm may be due to the excitation of excimers[7] of **1**. Such a phenomenon is observed to a lesser extent in D_2O based buffer (probably due to the higher viscosity of D_2O) and is absent at concentrations lower than 0.5 μmol dm^{-3} in H_2O buffer solution.

The emission spectra of **1** in buffers containing SDS [Figs. 1(*c*) and (*d*)] are much more intense than in buffer alone and are of a similar intensity in H_2O and D_2O solutions. This increase in quantum efficiency in aqueous micellar solution may be due to a longer excited state lifetime of **1** as a result of increased structural rigidity in the micelle and a decreased interaction with other components (*e.g.* oxygen and the buffer constituents) in solution.[8] We have not measured the fluorescence lifetime of **1** under the conditions given in Fig. 1. However, the lifetime of other polyaromatic molecules (*e.g.* pyrene) has been reported[9] to be about 50% longer in SDS than in water alone.

Fig. 2 shows a titration of fluorescence intensity of **1** with SDS concentration in water or D_2O alone, and in buffer-H_2O or buffer-D_2O solutions. A slope change occurs in all cases on going from the higher to the lower SDS concentrations. As fluorescence of **1** will be sensitive to local phenomena we interpret the break in slope as being due to the critical micelle concentration (cmc) of SDS. In fact, in agreement with literature findings[4,5] the apparent cmc was found to be lower in D_2O (6.4 mmol dm^{-3}) than in H_2O (7 mmol dm^{-3}) solution. These indirectly obtained values are slightly lower than ones reported from conductometric measurements.[5] This difference is consistent with the solubilisation of **1** in SDS being affected both by electrostatic and hydrophobic interactions. The introduction of the ionic buffer constituents to solution has a significant effect on both the fluorescence intensity of **1** and the apparent cmc values (Fig. 2). Under these conditions fluorescence is increased and the cmc is decreased to values close to 3 mmol dm^{-3}. Once again, the cmc in D_2O-based buffer is lower than that in H_2O-based buffer solution. The value of the cmc in water based buffer is of the same order of magnitude as that (2.6 mmol dm^{-3}) reported by Brito and Vaz[9] in phosphate buffer (pH 7.5) using the fluorescent probe *N*-phenyl-1-naphthylamine.

MECC is a popular[10–12] mode of operation of capillary electrophoresis. Here separation of analytes is achieved using an ionic micellar solution moving in an electric field. This technique can be used successfully for the resolution of both charged and neutral molecules. In the case of SDS at concentrations of higher than the cmc, micellar aggregates

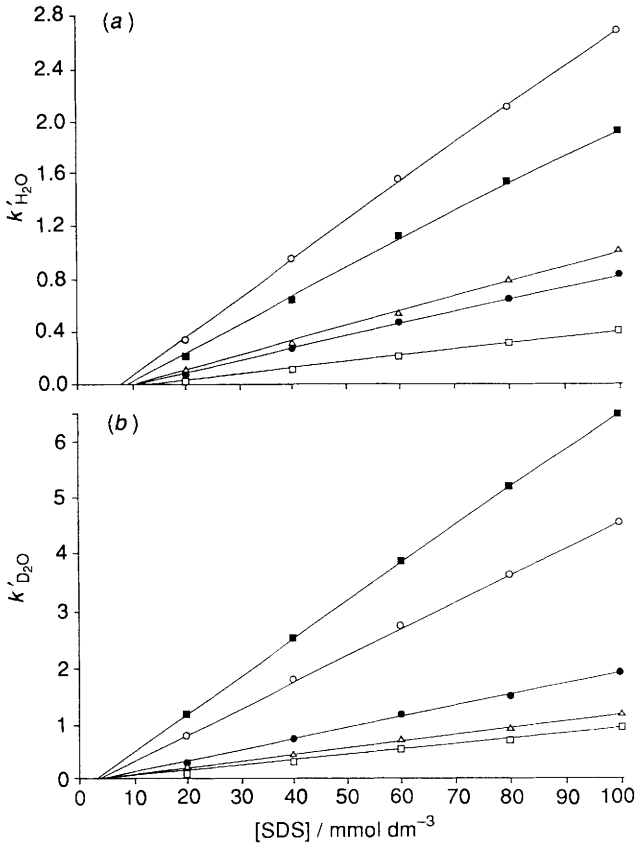

Fig. 4 Plots of (*a*) k'_{H_2O} and (*b*) k'_{D_2O} *vs.* SDS micelle concentration. (□) phenol; (△) 3-methoxyphenol; (●) 4-methoxyphenol; (○) 4-chlorophenol; and (■) 4-bromophenol.

move towards the negative electrode primarily under the influence of the electroosmotic velocity. Thus, molecules that partition slowly into the micelle migrate faster than those with higher partition coefficients. In a similar manner to reversed-phase high-performance liquid chromatography (HPLC) the order of elution is related to hydrophobicity.

$$k' = t - t_o/[(1 - t/t_{mc})\, t_o \qquad (1)$$

The capacity factor k' in MECC is calculated by eqn. (1), where t, t_o and t_{mc} are the migration times of analyte, of a solute excluded from the micelle and that of the micelle, respectively. Water or D_2O can be used to determine the value of the migration of the electroosmotic front, t_o, due to a change in refractive index. Sudan III and Sudan IV have been used to identify the migration time of the SDS micelle.[9,13] In our experience these dyes are inconvenient tracers as they are very insoluble in aqueous media unless methanol is added as co-solvent. It appears that the presence of methanol can cause disruption of the micelles, affects the stability of the current and produces inaccurate values for t_{mc}. Moreover, the absorbance of these dyes at low concentrations is not sufficiently high to enable the peak to be distinguished from a noisy baseline. Finally, as 'Sudan' type compounds are 'cancer suspect agents' it is undesirable to use them routinely.[14]

Having obtained ample evidence about the strong inclusion of halofantrine **1** in SDS micelles from the fluorescence studies, we have used this compound to measure t_{mc} and hence k' values of a number of phenols [(1), phenol; (2), 3-methoxyphenol; (3), 4-methoxyphenol; (4),4-chlorophenol; (5), 4-bromophenol] that are largely unionised at pH or pD around 7. Fig. 3(*a*) and (*b*) compare electropherograms obtained in H_2O and D_2O based buffer solutions. Migration times are longer in D_2O solution due to lower electroosmotic flow. Besides the better resolution obtained in heavy water Fig. 3 demonstrates the usefulness of halofantrine as a micelle marker. In fact the migration time of this molecule has been found[15] to be slightly longer than that of Sudan III again verifying the tighter inclusion of halofantrine in SDS micelles.

Figs. 4(*a*) and (*b*) show plots of the variation of k' with SDS concentration. Within the concentration range of SDS studied linear plots are obtained which intersect the SDS axis close to one point, related to the cmc of SDS.[12] As expected, the average cmc (~8 mmol dm^{-3}) obtained in H_2O-based buffer (pH 7.01) solution is higher than that (~3 mmol dm^{-3}) obtained in D_2O solution of the same acidity. The latter cmc value is very close to that determined from fluorescence measurements reported earlier. It is difficult to explain the difference in cmc values obtained by the two techniques in H_2O-based buffer. This is in part due to the scatter obtained in determining cmc values by MECC.[16] Moreover, as the temperature inside the capillary in the H_2O-based buffer is expected to be higher than that in the D_2O-based solution of the same pH or pD this may have a greater influence on the cmc in the former case.

In conclusion, we have shown by fluorescence studies that halofantrine is very strongly bound to SDS micelles. The intense UV absorbance and fluorescence intensity shown by this molecule makes it an ideal marker for the migration of SDS micelles in an electric field. The behaviour of halofantrine in other surfactants commonly used in MECC (*e.g.* cholic acid and taurodeoxycholic acid) is presently being investigated, especially that it has been reported[17] recently that such surfactants are superior to SDS for the resolution of analytes of higher hydrophobicity.

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