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Media Influence on the Enhancement of the Fluorescence of Berberine Hydrochloride

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Summary. Berberine hydrochloride is an alkaloid with little or no fluorescence in water. In sodium dodecylsulfate solutions, the fluorescence intensity of this compound is enhanced several folds by ion-pairing with the anion of the surfactant. The enhanced fluorescence intensity reaches a maximum at a surfactant concentration of $4 \cdot 10^{-3}\,M$ and then decreases to a constant value at the critical micelle concentration and beyond. At concentrations near the maximum, a calibration sensitivity of $3.23 \cdot 10^{6}/M$ was obtained. In addition, a good linear dynamic range and a low limit of detection ($4 \cdot 10^{-5}$ and $1.5 \cdot 10^{-7}\,M$, respectively) were determined. This observation indicates that this surfactant medium could be effectively used in fluorometric trace analysis of berberine hydrochloride. It was also observed in this work that solvents of low dielectric constant tend to stabilize this compound and thereby enhance its fluorescence.

Keywords. Alkaloid; Berberine; Fluorescence spectroscopy; Surfactant; Dielectric constant.

Introduction

Berberine hydrochloride (**1**) whose structure is shown in Fig. 1 is an alkaloid derived from *Berberis aristata*. **1** has been found effective in the treatment of cholera and other gastrointestinal disorders. It is known to be a popular constituent in most oriental pharmaceutical preparations because of its antimicrobial activity [1–4]. **1** and some of its derivatices have also been found to be effective in the treatment of tuberculosis, whose appearance and recent resurgence in different parts of the USA has been associated with AIDS and HIV patients (Ref. [5] and references cited therein). The upsurge in the incidence of tuberculosis has spurred active research in laboratory synthesis, extraction development, and characterization of **1** and its derivatives [4]. The analytical techniques for the determination of **1** have been discussed in the lieterature [6–12], and because of its importance in the treatment of tuberculosis, more techniques have been developped over the last decase [13–20]. In addition, there are electroanalytical methods for the determination of **1** [21–24]. The importance of this compound demands a critical development of other facile and relatively simple complimentary techniques, including fluorescence that is known to be most suitable in the determination of trace fluorescent chemicals and biological compounds. **1** is virtually nonfluorescent; fluorescence techniques for berberine characterization have been achieved to some extent by indirect methods including its entrapment in a sol-gel matrix and

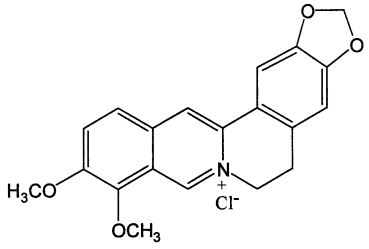

Fig. 1. Structure of berberine hydrochloride (**1**)

subsequent determination *via* quenching [25]. We report in this work the influence of solvent and surfactant on the fluorescence enhancement of **1**.

Results and Discussion

Figure 2 shows the fluorescence spectra of **1** obtained in sodium dodecylsulfate (*SDS*) solution and in water. The spectrum obtained in *SDS* solution is about 20 times more intense than that measured in water. *SDS* is a surfactant with a negatively charged polar head. It is assumed that the fluorescence enhancement of **1** is due to ion-paring of the berberinium ion and the anion of *SDS*. This ion-paring phenomenon has been used previously in the analysis of **1** and other quaternary ammonium ions [8–10, 20, 26].

SDS forms micelles in water; it was therefore decided to investigate the relative fluorescence of **1** at different concentrations of *SDS*. Figure 3 shows the profile of the observed relative intensity of **1** as the concentration of *SDS* increases. A maximum intensity at a concentration of **1** of $2.0265\times10^{-5}\,M$ was observed when the *SDS* concentration reached about $4\times10^{-5}\,M$. Thereafter, the intensity decreased to an almost constant value starting at the critical micelle concentration (CMC) of *SDS*

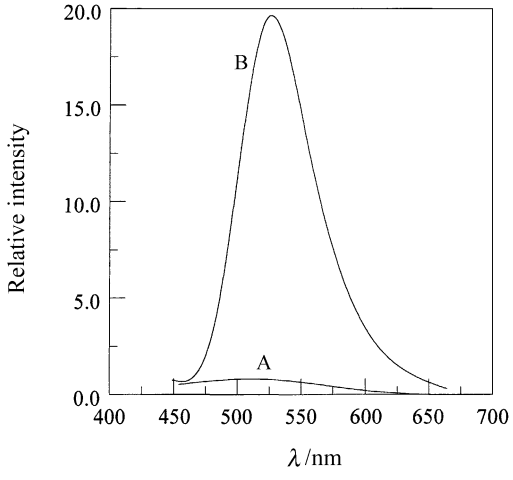

Fig. 2. Plot of the relative intensity of the fluorescence of $6.6\times10^{-5}\,M$ **1** in H_2O (A) and in 1.622 m*M* *SDS* (B)

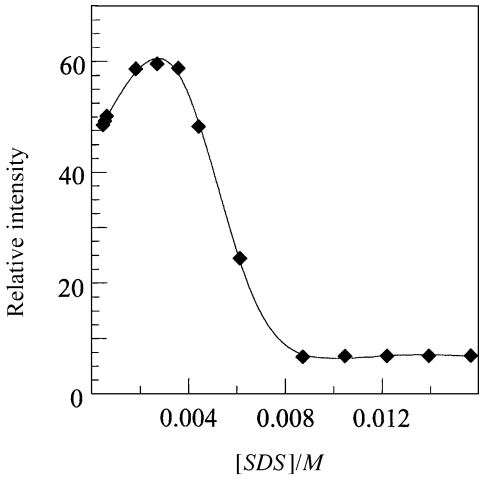

Fig. 3. Plot of observed relative fluorescence intensity of 2.0265×10^{-5} *M* **1** *vs.* [*SDS*]

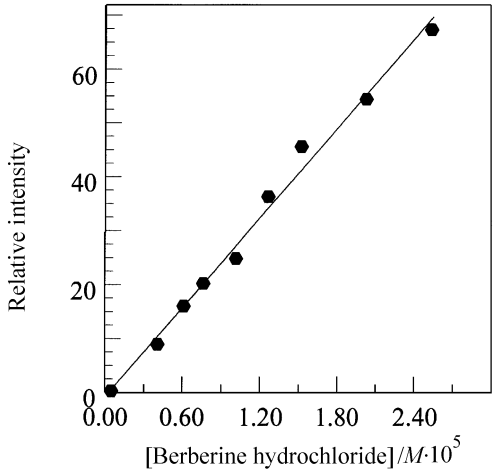

Fig. 4. Plot of observed relative fluorescence intensity of 3.484 m*M SDS vs.* [**1**]

that is known to be 8×10^{-3} *M* [27]. The onset of surfactant aggregation is marked by the decrease of the fluorescence intensity of **1**, and upon complete micelle formation the intensity becomes virtually independent of *SDS* concentration. It may be inferred that this decrease is due to a complete incorporation of **1** into the formed micelle. This observation, which is not an uncommon characteristic with fluorescent molecules in micellar solutions, has been used to determine the CMC of surfactants [28, 29].

In order to fully understand the behavior of **1** in the *SDS* micellar solution, concentration dependence studies of this compound were conducted at two surfactant concentrations: below and above the CMC. The point chosen before the CMC was that near the maximum. Figure 4 shows a typical plot of relative fluorescence intensity *versus* the concentration of **1** at an *SDS* concentration of 3.484×10^{-3} *M*. As can be seen, a linear plot was obtained up to a concentration

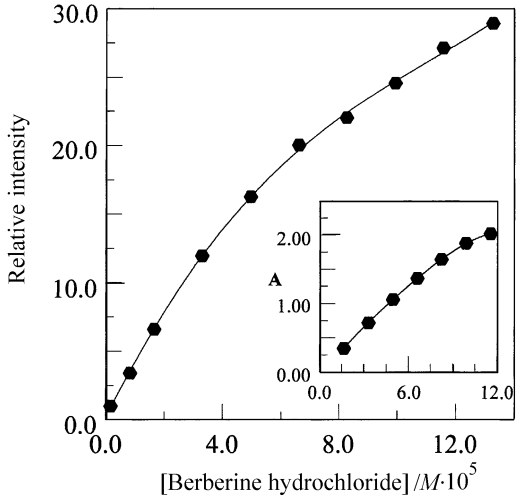

Fig. 5. Plot of relative fluorescence intensity *vs.* [**1**] at an *SDS* concentration of $1.622\times10^{-2}\,M$ and in H_2O (inset)

of $2.41\times10^{-5}\,M$ with a calibration sensitivity of $3.23\times10^{6}\,M$. On the other hand, Fig. 5 shows a plot of observed relative fluorescence intensity *versus* concentration of **1** at an *SDS* concentration above the CMC ($1.622\times10^{-2}\,M$). This plot shows that beyond a $4\times10^{-5}\,M$ solution of **1** the correlation is not linear. Analysis of the observed data shows that the dynamic range, which is the concentration between the limit of quantitation and the limit of linearity [30], lies between 5×10^{-7} and $4\times10^{-5}\,M$ with a limit of detection (c_L) of $1.5\times10^{-7}\,M$. This value of c_L is consistent with literature data for the determination of **1** using a different technique [18]. A measurement of 18 blanks was used to determine the limit of detection using the relation $c_L = 3s_{bl}/m$ where s_{bl} is the standard deviation of the blank intensity and m is the slope of the calibration plot of Fig. 4.

In Fig. 5, the nonlinearity of fluorescence intensity at increasing concentration of **1** is shown; the inset presents the absorbance of **1** with increasing concentration. The plot does not obey *Lambert-Beer's* law and shows a deviation exactly at the same concentration of $4\times10^{-5}\,M$ as in the fluorescence experiment. It is believed that this nonlinearity is due to interaction of the berberinium ion with water leading to some aggregation.

Analytical utility

Notwithstanding the nonlinearity of the plot in Fig. 4, the sensitivity together with the relatively good linear dynamic range and the low limit of detection shows that **1** could be effectively determined in a *SDS* solution with good analytical result.

Solvent effect

The fluorescence of **1**, as stated earlier and shown in Fig. 2, is very low in aqueous solution and therefore of no analytical utility. However, this compound fluoresces

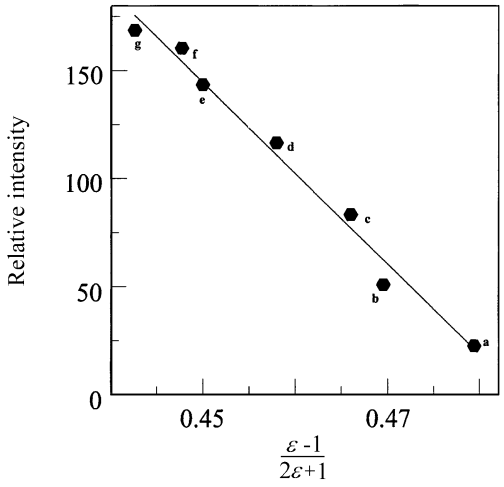

Fig. 6. Plot of relative fluorescence intensity *vs*. solvent parameter; a: methanol, b: ethanol, c: 1-propanol, d: 1-butanol, e: 1-pentanol, f: 1-hexanol, g: 1-heptanol

Table 1. Dielectric constant, solvent parameter, and relative fluorescence intensity of **1** in different solvents

	ε	$\frac{\varepsilon-1}{2\varepsilon+1}$	Relative intensity
Methanol	32.63[a]	0.4774	22.40
Ethanol	22.60[a]	0.4675	50.80
1-Propanol	20.33	0.4640	83.20
1-Butanol	16.52[a]	0.4559	116.50
1-Pentanol	13.9	0.4479	143.20
1-Hexanol	13.3	0.4456	160.30
1-Heptanol	12.1[b]	0.4405	168.50

[a] Adjusted to 25°C using the approximate relation on page 133 of Ref. [31];
[b] value at 22°C

appreciably when dissolved in other solvents [6, 11]. Figure 6 shows the relative fluorescence intensity of **1** in different alcohols plotted as a function of the respective solvent parameter $(\varepsilon-1)/(2\varepsilon+1)$ where ε is the solvent dielectric constant [31]. Table 1 lists the solvents used together with their static dielectric constant with which the solvent parameters were calculated. As can be seen from Fig. 6, the observed fluorescence intensity varies inversely linearly with the dielectric constant and the solvent parameter. Solvents of high dielectric constant appear to increase the ionization of the quaternary ammonium ion, thereby destabilizing the electronic resonance of the compound with a resultant effect of low fluorescence. This fact may account for the very marginal fluorescence observed in water, a solvent of high dielectric constant.

Conclusion

It has been shown in this work that **1** exhibits very low fluorescence in water. Its fluorescence is enhanced by ion-paring with *SDS* in a surfactant solution. At the CMS of this surfactant, the observed fluorescence intensity of **1** decreases to a minimum and becomes independent of the surfactant concentration. **1** has a dynamic linear range between 5×10^{-7} and $4\times10^{-5}\,M$, a limit of detection of 1.5×10^{-7}, and a calibration sensitivity of $3.23\times10^{6}/M$ in *SDS* solution and can, therefore, be quantitatively analyzed in this medium.

Experimental

Materials

1 of 99% purity was obtained from Aldrich. Electrophoresis grade sodium dodecylsufate and reagent grade alcohols were obtained from Fisher Scientific. All chemicals were used as received.

Method

All fluorescence measurements were performed using a Perkin Elmer luminescence spectrophotometer model LS 50 B. Unless otherwise stated, all measurements were executed excitation and emission wavelengths of 382 and 520 nm. Absorptiometric experiments were conducted with a Cary spectrophotometer model 1E. The absorption maximum observed at 347 nm was used for all analyses. All solutions were prepared with triply distilled and deionized H_2O obtaind by a Reagent Grade Water System deionizer from Photronix. All experiments were conducted at room temperature ($25\pm0.2^{\circ}C$).

Acknowledgements

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