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Fluorescence of berberine in microheterogeneous systems

Ariel N. Colina, Marta S. Díaz, María Isela Gutiérrez *

Departamento de Química, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, 9000-Comodoro Rivadavia, Argentina

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ABSTRACT

Spectral properties of the alkaloid berberine were studied in micellar solution and microemulsions based on anionic sodium dodecyl sulfate, cationic cetyltrimethylammonium bromide and nonionic Triton X-100 surfactants. Absorption and fluorescence emission spectra were determined. For screening the influence of type and concentration of micelles on the fluorescence of berberine a 3^2 full factorial design was used. Higher responses were obtained when berberine was dissolved in sodium dodecyl sulfate micelles 0.01 M. Comparative results of fluorescence quantum yields (Φ_f) reveal that the highest values ($\Phi_f \geq 0.01$) were observed in microemulsions. In the microheterogeneous systems investigated the most probable location of berberine is the micellar interfacial region.

1. Introduction

Berberine (**B**, Scheme 1) is an alkaloid found in roots and barks of *Berberis* species and exhibits diverse applications due to its pharmacological activity [1–7] and to its fluorescence as a probe molecule in biological and analytical studies [8–11]. The spectroscopic properties of **B** have been found to be highly dependent on the media [12,13] and its solvatochromism makes **B** a potential probe for polarity and also for hydrogen bonding properties of the local microenvironment. Investigation of **B** in organized media is an active area of research [14–22]. Although the fluorescence of **B** has been studied in homogeneous [12,13] and micellar media [15,17,21] no data are available in microemulsions, which have attracted considerable interest in recent years because they can improve the oral bioavailability and therapeutic effect of **B** [23,24].

The interaction of molecular probes with microheterogeneous systems that resemble many biological and chemical structures in nature, such as micelles, vesicles or liposomes usually results in dynamic binding, and the spectroscopic properties of the probe depend on its location. The distribution of molecules in these microheterogeneous environments is markedly influenced by the hydrophobic/hydrophilic character of the surrounding medium and by the presence of electrostatically charged interfaces. The understanding of the interactions between ionic fluorescent probes such as **B**, and charged surfaces is also of interest in several applications, which may include the characterization of drug-delivery systems and the micelles enhanced emission detection in analytical techniques.

Here we present results concerning the influence of micelles and microemulsions on the spectroscopic properties of the singlet excited state of **B**. Initially, the influence of nature and concentration of surfactants on the fluorescence properties of **B** has been investigated by means of a complete factorial design, which allow the quantification of the main effects of several factors, and the interactions between them, with the same precision but with a smaller sample size, compared to the traditional step-by-step approach [25]. Efficient experimental designs, based on multivariate methods have been applied to study various types of problems, for example in relation with **B**, the optimum conditions to produce **B** treated polyamide substrates was successfully studied applying a 2^4 central composite design [26]. In this work, a set of experiments was planned according to a 3^k full factorial design to optimize experimental conditions for detection of **B** by fluorescence. The selected response was fluorescence intensity (I_f) and solutions of three different kinds of surfactants were employed: anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethylammonium bromide (CTAB) and nonionic Triton X-100 (TX). In order to further explore the effect of the media on the spectroscopic properties of **B**, the fluorescence quantum yields have been determined in micellar media in the conditions obtained from the factorial design, and in different types of microemulsions.

2. Experimental

2.1. Materials

Berberine chloride, fluorescein and quinine sulfate from Sigma Chem. Co., sodium acetate from Baker, sodium hydroxide from Merck and the following surfactants: sodium dodecyl sulfate,

* Corresponding author. Tel./fax: +54 297 4550 339.
E-mail address: isela@unpata.edu.ar (M.I. Gutiérrez).

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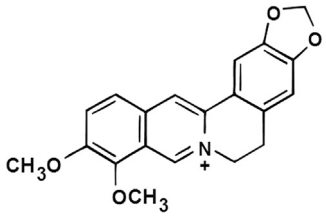

Scheme 1. Chemical structure of berberine (**B**).

Table 1
Full factorial experiment design (3^2)

Standard order [a]	Run order[b]	X_A	X_B	y_1[c]
1–10	3–6	SDS	0.0001	0.5704
2–11	17–18	SDS	0.0010	1.3028
3–12	10–16	SDS	0.0100	6.2026
4–13	11–12	TX	0.0001	0.5288
5–14	5–15	TX	0.0010	0.8825
6–15	7–14	TX	0.0100	2.8815
7–16	8–9	CTAB	0.0001	0.4148
8–17	1–13	CTAB	0.0010	0.9211
9–18	2–4	CTAB	0.0100	2.5847

[a] Standard order.
[b] Experimental order.
[c] Fluorescence intensity.

cetyltrimethylammonium bromide and Triton X-100 from Merck were used as received. Prior to their use, it was checked that the surfactants do not contribute to either absorption or fluorescence in the region of interest. Doubled distilled water and the organic solvents cyclohexane (CH, U.V.E.), 1-butanol (BuOH, AnalaR) and acetic acid (Ac, Merck) were used.

2.2. Experimental design

The experiments were carried out considering a 3^2 (three levels, two factors) full factorial design, consisting of 18 experiments (two runs for each of the 9 experiments). The selected factors were type of micelles (X_A) and its concentration (X_B). Fluorescence intensity (y_1) was chosen as dependent output response variable. The range of variation (experimental region) was selected on the basis of previous experiments, for X_A (type of surfactant): anionic (SDS), cationic (CTAB) and nonionic (TX) (critical micellar concentrations (CMC): 8.3×10^{-3} M, 1.1×10^{-3} M and 2.6×10^{-4} M, respectively) [27,28] and for X_B (surfactant concentration): 0.0001 M (< CMC of the surfactants), 0.001 M and 0.01 M (> CMC). The variables X_A and X_B were coded to the levels 1 (low), 2 (intermediate) and 3 (high). The full factorial design, including the factors, their levels and the result from each test, is shown in Table 1.

2.3. Micellar media and microemulsions

For the determination of fluorescence quantum yields, micellar solutions contained 0.01 M SDS, CTAB or TX. The composition of the oil-in-water (o/w) and water-in-oil (w/o) in weight percent are given in Table 2 [27]. Stock solutions containing **B** were prepared in water with or without buffer. The pH was adjusted to 4.0 by adding a sodium acetate buffer with acetic acid. The final **B** concentrations, around 10 μM, gave adequate emission intensities and ensured no multiple probe occupation of the micelles. The solutions were gently hand-shacked at room temperature.

Table 2
Composition in weight percent and refractive indices at 589 nm (n_D, 20 °C) of the microemulsions.

Microemulsions	Surfactant (%)	Cosurfactant (%)	Water (%)	CH (%)	n_D
o/w SDS	8	16	72	4	1.417
w/o SDS	8	16	7	69	1.360
o/w CTAB	8	16	72	4	1.362
w/o CTAB	8	16	7	69	1.418
o/w TX	28	7	62	3	1.385
w/o TX	8	16	7	69	1.418

2.4. Absorption and emission measurements

Ground state absorption spectra were measured at room temperature on an Agilent 8453E diode array spectrophotometer. Fluorescence spectra were measured at room temperature in air equilibrated solutions with a Jasco FP6200 spectrofluorometer. The corrected emission spectra were obtained by excitation at 400 nm (for CTAB or TX) or 450 nm (for SDS) with an excitation and emission slit width of 5 nm. Optically matched solutions of the sample and reference were used. Fluorescence quantum yields were determined using quinine sulfate in 0.1 M H_2SO_4 (Φ_S=0.546) [29] or fluorescein in ethanol (Φ_S=0.97) [30] as the reference compound. Fluorescence quantum yields of **B** (Φ_f) were determined according to Eq. (1) [29]

$$\Phi_f = \frac{A_S F_B n_B^2}{A_B F_S n_S^2}\Phi_S \quad (1)$$

were A, F and n denote absorption at the excitation wavelength (λ_{exc}), integrated area underneath the fluorescence emission spectrum corrected after solvent blank subtraction and refractive index of the solvent, respectively. The absorbance of **B** was always < 0.1 at excitation wavelength in a 1 cm cell. All measurements were done by triplicate (n=3) and reproducible results were obtained. All measurements were made at 23 ± 2 °C.

2.5. Data analysis

Design and analysis of the factorial experiments and the correlations were carried out on a personal computer with MINITAB™ (Minitab Inc) Release 14.20 statistical package. Analysis of variance (ANOVA) was used to analyze the results employing MINITAB software. Various statistical data (standard error of estimate, sum of squares of the errors, F-statistics, p-value) were examined. Schemes and spectra were made with ACD/ChemSketch (Freeware) and SciDAVis 0.2.4 software, respectively.

3. Results and discussion

Variables affecting the fluorescence of **B** in micellar media were initially studied by a full factorial design. Once the experiments were carried out in the conditions listed in Table 1, analysis of variance (ANOVA, Table 3) was used to analyze how the experimental factors affect I_f. Both F-tests and p-values (< 0.05) showed that the effect of each factor and their interactions were all statistically significant. In order to gain insights about the effect of each variable on the I_f of **B**, it is helpful to look at the main effect plots of Fig. 1. The presence of the cationic surfactant CTAB below its CMC gives the lowest I_f. On the other hand, the anionic surfactant SDS beyond its CMC appears to be the suitable condition for the detection of **B** by fluorescence. Additionally, the effect of one variable depends on the setting of the other. Fig. 1 (inset) shows the plot of the interactions between main variables. There are significant two order interactions, indicated by non-parallel