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Use of micellar media for the fluorimetric determination of ellipticine in aqueous solutions[1]

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Abstract

Ellipticine is a pyridocarbazole alkaloid with interesting antitumour activity. Use of neutral ellipticine is hampered by its very low water solubility and therefore this compound has been administered as a salt; however, nitrogen quaternization modifies the antitumour properties of ellipticine. Potential alternatives to quaternization include the use of cyclodextrins, and also the use of micellar media. The latter possibility is explored in this work as an analytical tool. The results obtained with model anionic (SDS), cationic (CTAB) and neutral (Brij-35) surfactants are described. Fluorimetric analysis shows that ellipticine solubilizes completely in the presence of all these compounds, as a result of its aromatic, planar structure. The use of micellar media considerably increases the slopes of the calibration curves with improved correlation coefficients (e.g. 0.8904 in water and 0.9982 with SDS). Micellar media also modify proton transfer processes, as a consequence of the apolar environment of the micellar phase. Deprotonation of ellipticine is hampered in SDS because of the relationship between this process and the surface charge of the micelles. Finally, fluorescence quenching in micellar media has been studied, it being found that surfactants provide protection towards this phenomenon.

Keywords: Alkaloids; Fluorimetry; Micellar solutions

1. Introduction

Ellipticine and other pyridocarbazoles are usually classified as indole alkaloids [1] and are important owing to their antitumour activity due to inhibition of DNA replication and RNA transcription, both "in vivo" and "in vitro" [2]. Ellipticine, in particular, has found clinical application in advanced breast cancer, mieloblastic leukemia and solid tumors [3].

Ellipticine has a basic, pyridine-like nitrogen, which protonates at pH < 7.4 [4], and a pyrrolic nitrogen which is ionized under strongly basic conditions. Ellipticine is soluble in water only as a cationic species, which is less active than the

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neutral form [5] and is not absorbed through cellular membranes [6]. Inclusion of ellipticine into γ-cyclodextrin [4] or modified β-cyclodextrins [7] has been suggested as a technique for achieving aqueous solubility of the ellipticine neutral species.

Ellipticine shows a high native fluorescence [8] which, similar to that of carbazole, is pH-dependant and therefore high performance liquid chromatography (HPLC) with fluorimetric detection is an excellent method for ellipticine quantitation [9,10]. Due to the current interest in the use of micellar media for the improvement of the selectivity of the separation and the sensitivity of the fluorimetric detection [11] in HPLC [12–14], the effects of micellar media on the native fluorescence of ellipticine and their influence on the acid–base equilibria as well as the protection they afford against fluorescence quenching by bromide anion were studied.

2. Experimental

2.1. Apparatus and reagents

UV–visible spectra were obtained with an automatic double beam Kontron Uvikon 810 spectrophotometer equipped with a model 800 printer–plotter. Uncorrected fluorescence spectra were carried out with a Perkin-Elmer MPF-2A fluorimeter (Xenon lamp, 150 W). All the reagents and solvents were analytical grade and were used without further purification. Ellipticine was purchased from Fluka and the surfactants sodium *n*-dodecyl sulfate (SDS), *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (CTAB) and polyoxyethylenlaurylether (Brij-35) were from Merck. Water was bidistilled and deionized (Milli-Q) prior to use.

2.2. Procedures

Micellar solutions were prepared in water at concentrations of 4×10^{-2} M for SDS (CMC $= 8.1 \times 10^{-3}$), 5×10^{-3} M for CTAB (CMC $= 9.2 \times 10^{-4}$ M) and 5×10^{-4} M for Brij-35 (CMC $= 9.0 \times 10^{-5}$) [15]. The solutions were degassed for 30 min under ultrasound and stabilized for 24 h at room temperature.

A 1×10^{-3} M ethanolic stock solution of ellipticine was prepared. Aliquots of 50 μl were taken from this solution and evaporated in vacuo at room temperature. Appropriate volumes of micellar solution were added to the film thus formed, so that the final concentration of ellipticine was 1×10^{-6} M. These solutions were magnetically stirred for 1 h in a thermostatted water bath (20 ± 2°C). Quantitative analyses of ellipticine were carried out using the same procedure but varying the final concentrations in the micellar solution. Acid–base equilibria in micellar media were studied employing the procedure described above for preparing the ellipticine/surfactant solutions. Aliquots of 10 μl of NaOH (1×10^{-2} M, 1×10^{-1} M and 1 M) were added to the sample ellipticine/surfactant solution and the same procedure was employed for the addition of hydrochloric acid (1×10^{-2} M, 1×10^{-1} M and 1 M). Fluorescence quenching of ellipticine was studied using bromide ion as quencher. Aliquots of a NaBr solution were added to the different ellipticine/surfactant solutions. The final concentrations of bromide ion were varied from 0 to 0.5 M. The ionic strength of the solutions was adjusted to a final value of 1 KCl or NaCl, depending on the surfactant.

3. Results

Ellipticine is a compound with a significant native fluorescence although its emission properties are not clearly described. Thus in organic solvents (Fig. 1a) or DMSO [8] it exhibits a emission band with $\lambda_{em} = 430$ nm ($\lambda_{ex} = 294$ nm). In water (Fig. 1a) it presents a band with maxima at 350 and 360 nm and a broad band with a maximum at 520 nm ($\lambda_{ex} = 304$ nm). The fluorescent emission at 520 nm in water solutions has already been described [4,16] and it has been attributed to the cationic species of ellipticine which predominates in aqueous solutions at a pH value below 7.4. Fig. 1b and Fig. 1c show that the addition of NaOH or HCl to ethanolic or aqueous solutions of ellipticine changes the emis-

sion spectra. Thus in ethanolic solution (10^{-6} M) the emission band at 430 nm can be observed, and after addition of HCl this band disappears to produce the species responsible for the emission at 520 nm, together with a very weak fluorescence at 350–360 nm (Fig. 1b). Addition of NaOH to the ethanolic solutions causes only an increase in the emission in intensity at 430 nm. In water solutions of ellipticine, NaOH additions shift the fluorescent emission from 520 nm to 430 nm together with a decrease in the emission intensity at 350–360 nm which is present in water solution (Fig. 1c). Addition of HCl to the aqueous solution of ellipticine produces an increase in the fluorescent emissions at both 350–360 nm and 520 nm (Fig. 1c).

The coexistence of different equilibria among the neutral and zwitterionic together with the cationic and anionic species of ellipticine can be presumed to be similar to those for other alkaloids such as β-carboline derivatives [17]. These equilibria are presented in Scheme 1. Acid–base equilibria can occur in both ground and excited states. However, the acidity of excited states differs remarkably from that of the ground states. In the case of ellipticine the presence of two different forms, i.e. cationic and zwitterionic (Fig. 1a), is observed by spectrofluorimetry at the same pH value and these species originate in the ground state because they present different excitation spectra ($\lambda_{ex} = 292$ nm neutral and zwitterionic forms, $\lambda_{ex} = 305$ nm cationic form). Nevertheless cationic, zwitterionic and neutral forms present different emission spectra depending on the pH value, and consequently the appearance of these species in very low concentrations can be sensitively detected by spectrofluorimetry.

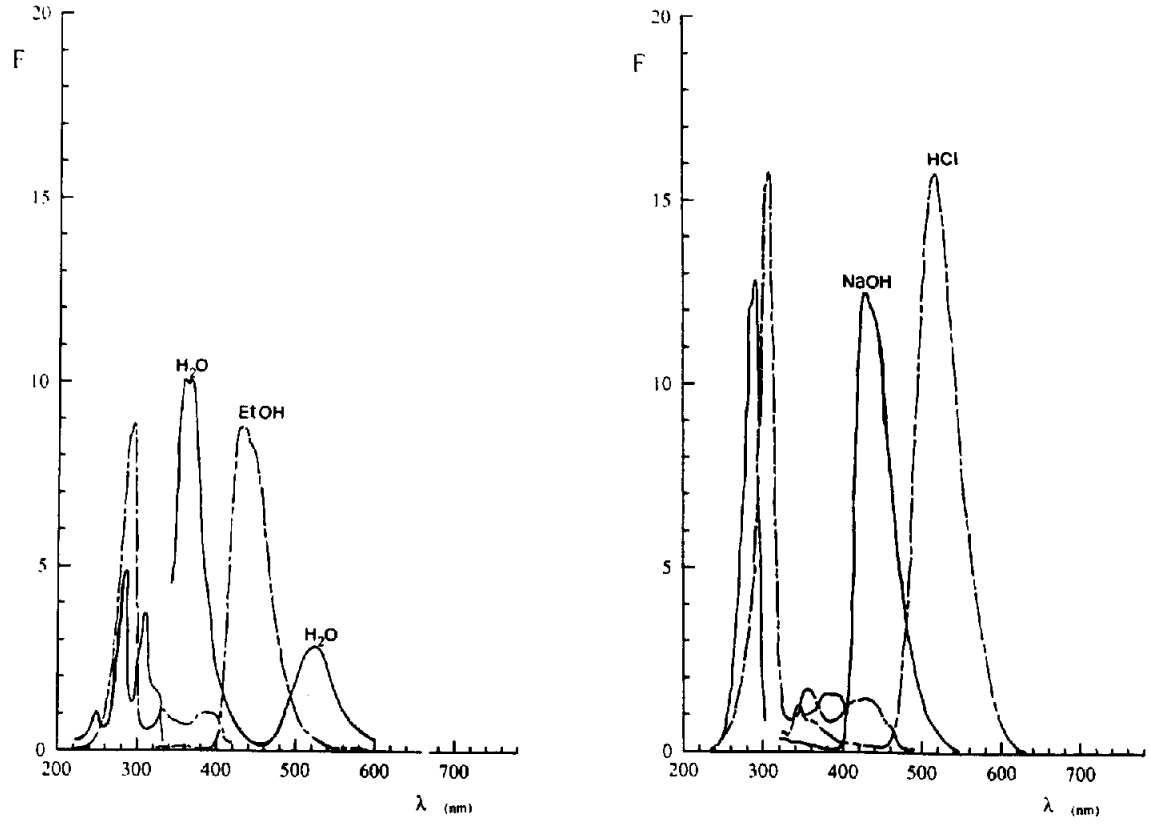
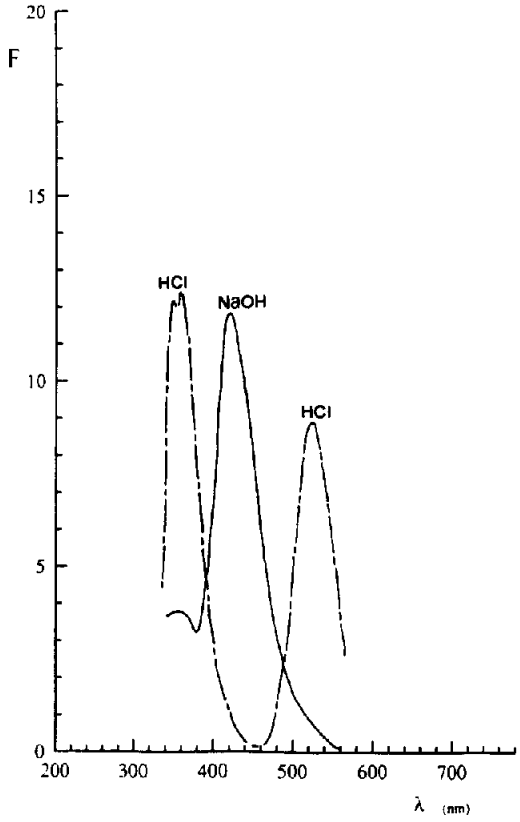


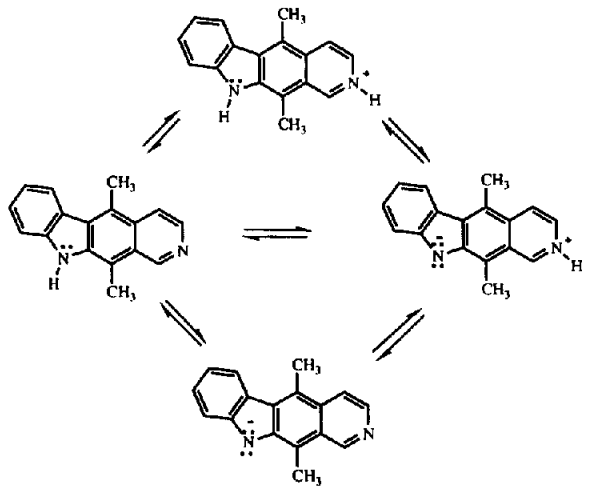
Fig. 1. (a) and (b).



(c)

Fig. 1. (a) Uncorrected excitation and fluorescence emission spectra of ellipticine ($C = 1$ μM) in water ($\lambda_{ex} = 305$ nm, $\lambda_{em} = 350$ nm) and ethanol ($\lambda_{ex} = 294$ nm, $\lambda_{em} = 433$ nm). Fluorescence intensity for aqueous solution is 81 times lower than for ethanol. F: fluorescence intensity in arbitrary units; λ: wavelength. (b) Uncorrected excitation and fluorescence emission spectra of ethanol solutions of ellipticine ($C = 1$ μM) with NaOH ($\lambda_{ex} = 294$ nm, $\lambda_{em} = 433$ nm) or HCl ($\lambda_{ex} = 307$ nm, $\lambda_{em} = 524$ nm). Fluorescence intensity for ethanol and HCl is three times lower than for ethanol and NaOH. F: fluorescence intensity in arbitrary units; λ: wavelength. (c) Uncorrected excitation and fluorescence emission spectra of aqueous solutions of ellipticine ($C = 1$ μM) with NaOH ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 430$ nm) or HCl ($\lambda_{ex} = 305$ nm, $\lambda_{em} = 524$ nm). F: fluorescence intensity in arbitrary units; λ: wavelength.

Surfactants are frequently employed to solubilize hydrophobic compounds as well as to increase the fluorescence intensity of weakly fluorescent compounds because micellar solutions afford a microenvironment with lower polarity than water. Fig. 2 shows the excitation and



Scheme 1. Equilibria of the different species (neutral, zwitterionic and ionic forms) of ellipicine.

fluorescence emission spectra for ellipticine, in homogeneous (water, ethanol) and micellar solutions. The emission band at 520 nm ($\lambda_{ex} = 305$ nm) is due to the quaternized cationic species and predominates in aqueous solution but is absent in ethanol. Micellar solutions may contain both the cationic and neutral forms of ellipticine, depending on the nature of the surfactant. Thus, in the presence of CTAB and Brij-35 only the band at 440 nm is observed upon excitation at the wavelength corresponding to the neutral species ($\lambda_{ex} = 295$ nm). This band which is also observed in ethanolic solution can be explained by solubilization of ellipticine in the micelles with a lower polarity microenvironment than water. However, in the presence of SDS two bands appear at 350–360 nm (with a very weak intensity) and 520 nm (Fig. 2). This behaviour can be explained by taking into account that the negatively charged micellar surface of SDS can stabilize the cationic ellipticine species. All micellar solutions cause a very significant ($\approx 100\%$) increase in the fluorescence emission intensity and therefore improve the sensitivity in the fluorimetric detection of ellipticine. Table 1 shows the linear regression data for the ellipticine quantitation in the 1×10^{-7}–1×10^{-6} M range. The increase in the analytical sensitivity is proved by

the higher slopes of the lines corresponding to ionic surfactant solutions. Furthermore, the correlation coefficients are better than in water due to the nonlinear response associated with the low aqueous solubility of ellipticine.

Acid–base equilibria can be significantly modified by the environment of the ionizable compound [16–18]. The displacement of the emission bands has been studied at 440 nm (neutral form) and at 520 nm (protonated form) after addition of sodium hydroxide or hydrochloric acid to micellar solutions of ellipticine, observing that protonation and deprotonation are normally disfavoured in the micellar environment. Thus Fig. 3 shows the effect of HCl additions on the acid–base equilibria in micellar and aqueous solutions of ellipticine and, as can be observed, higher amounts of HCl are necessary to achieve protonation in the presence of CTAB or Brij-35 than in aqueous solution. In the presence of SDS, the emission spectrum obtained corresponds to the protonated ellipticine species ($\lambda_{em} = 520$ nm). Accordingly, no changes are observed if hydrochloric acid is added. This difference is due to the anionic nature of the micellar surface, leading to stabilization of the protonated form of ellipticine and also to its accumulation in the surface of the micelles, which explains the smaller increase in the fluorescence intensity with respect to CTAB and Brij-35. Similarly, addition of NaOH caused deprotonation at higher pH values in SDS environments than in water, which agrees with previous observations made by Schwaller et al., [16], and can be explained by taking into account that the negative charge of SDS micelles hampers the hydroxide ion approach. Sodium hydroxide addition had little effect on CTAB and Brij-35 solutions as a consequence of protection of the neutral ellipticine molecules ($\lambda_{em} = 440$ nm) inside micelles.

Fig. 4 shows the possible distribution of ellipticine among the bulk aqueous phase and the different micellar solutions studied and, with the

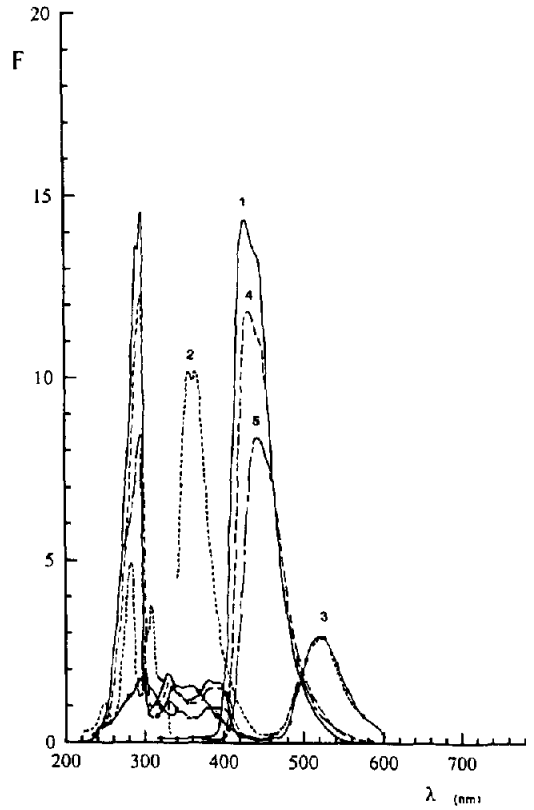


Fig. 2. Uncorrected excitation and fluorescence spectra of ellipticine ($C = 1$ μM) in different media: (1) ethanol; (2) water; (3) aqueous solution of SDS; (4) aqueous solution of Brij-35; (5) aqueous solution of CTAB. Excitation wavelength $\lambda_{ex} = 292–296$ nm for (1), (3), (4), (5) and 305 nm for (2). Emission wavelength $\lambda_{em} = 435–440$ nm for (1), (5) and (4) and 350 nm for (3) and (2). Fluorescence intensity for aqueous solution is 81 times, for SDS 9 times, and for Brij-35 and ethanol three times lower than for CTAB. F: fluorescence intensity in arbitrary units; λ: wavelength.

Table 1
Linear regression parameters obtained in the quantitative determination of ellipticine in the presence of different surfactants

Surfactant	Linear regression parameters of ellipticine		
	r[a]	b[b]	m[c]
H_2O[d]	0.8904	0.7275	6.158
SDS[d]	0.9982	1.1333	13.321
CTAB[e]	0.9951	3.0530	13.720
Brij-35[e]	0.9869	0.7750	4.750

[a] Correlation coefficient.
[b] Ordinate.
[c] Slope.
[d] $\lambda_{em} = 520$ nm.
[e] $\lambda_{em} = 440$ nm.

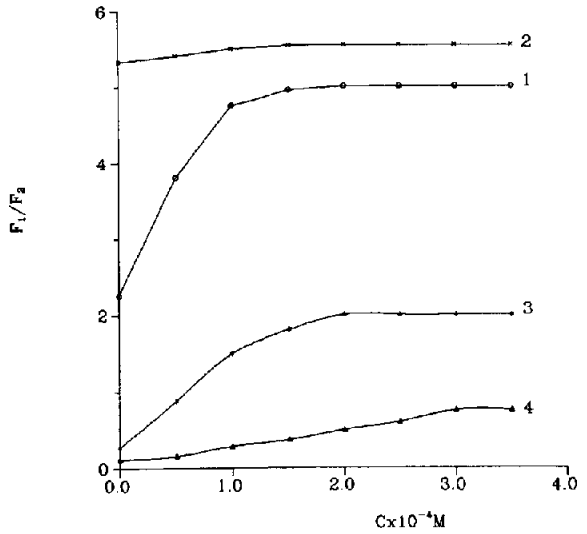


Fig. 3. Study of the effect of HCl addition on the acid–base equilibrium of ellipticine in the presence of different surfactants: (1) aqueous solution; (2) aqueous solution of SDS; (3) aqueous solution of Brij-35; (4) aqueous solution of CTAB. F_1/F_2: ratio of the fluorescence intensities (arbitrary units) at the emission maxima ($\lambda_{em} = 520$ nm and $\lambda_{em} = 440$ nm respectively). C: acid (HCl) concentration. F_1/F_2 for (2) must be multiplied by a factor of two.

aim of providing further data on the distribution of ellipticine in the micellar phases studied, fluorescence quenching by bromide anion was studied, in the presence of a micellar solution at constant ionic strength (Table 2). Good correlation coefficients were observed in all cases. Comparison of the Stern–Volmer constants (K) for micellar and aqueous solutions shows that SDS protects ellipticine from the quencher owing to accumulation of the ellipticine cation on the micellar anionic surface and therefore the negative charge of the micelles prevents bromide ions from coming close to ellipticine. In the case of CTAB and Brij-35 it is necessary to compare with the neutral form ($\lambda_{em} = 440$ nm) present in ethanol. In both micellar solutions the Stern–Volmer constants are lower than in homogeneous ethanolic solution. The Stern–Volmer constants in these surfactants should be compared with those measured in ethanolic solution because they show the same emission wavelengths, i.e. 440 nm (Brij-35, CTAB and ethanol), while in buffered aqueous solution the emission wavelength was 350 nm, which can be ascribed to the zwitterionic form. The Stern–Volmer constant obtained in Brij-35 was higher than those obtained in buffered aqueous solution, a behaviour that can be attributed to the different emission wavelengths (different ionic species) but also to a possible collisional quenching by bromide ion in this particular non-ionic micelle. However, the Stern–Volmer constants

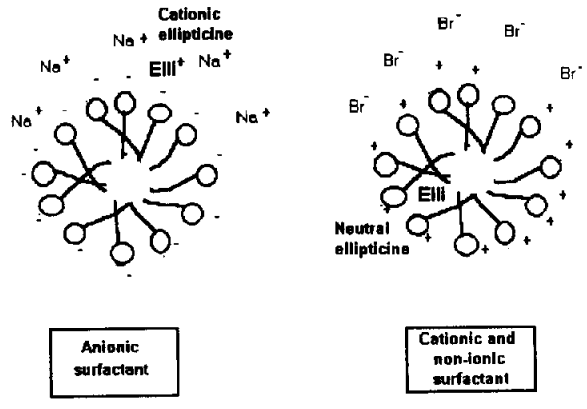


Fig. 4. Distribution proposed for ellipticine among the bulk aqueous phase and the different micellar media studied. Elli = neutral ellipticine; Ellli$^+$ = cationic ellipticine.

Table 2
Study of the quenching effect of bromide ion on the fluorescence of ellipticine in the presence of different surfactants. The ionic strength was adjusted with KCl to a value of 1.0 M. For SDS this adjustment was carried out with NaCl to a value of 1.0 M

Surfactant	Ellipticine Stern–Volmer parameters		
	r[a]	b[b]	$m(k)$[c]
H_2O[d]	0.9898	1.0113	0.7604
Buffer (pH 9.2)[e]	0.9976	1.0125	1.8214
Ethanol[f]	0.9688	0.9680	2.1000
SDS[d]	0.9777	0.9894	0.6324
CTAB[f]	0.9962	1.0068	0.9511
Brij-35[f]	0.9992	1.0085	1.8703

[a] Correlation coefficient.
[b] Ordinate.
[c] Slope (Stern–Volmer constant).
[d] $\lambda_{em} = 520$ nm.
[e] $\lambda_{em} = 350$ nm.
[f] $\lambda_{em} = 440$ nm.

for both surfactants are greater than those observed in SDS and they can be attributed to the different emission intensities at 520 nm and 440 nm.

In summary, surfactants improve the sensitivity of fluorimetric determinations of ellipticine. Distribution of the analyte into the micellar phase is drastically dependent on acid–base equilibria. Protonation and deprotonation of ellipticine are hampered by the presence of micelles. Micellar environments efficiently protect ellipticine from the quenchers.

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