

Role of curcumin on the determination of the critical micellar concentration by absorbance, fluorescence and fluorescence anisotropy techniques

Satyajit Mondal, Soumen Ghosh *

Centre for Surface Science, Department of Chemistry, Jadavpur University, Kolkata 700 032, India

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ABSTRACT

Curcumin ($C_{21}H_{20}O_6$) is a natural antioxidant which has a wide range of physiological and pharmacological actions. Here, first time this is employed for the determination of the critical micellar concentration (cmc) of both ionic and nonionic surfactants using the measurements of UV absorption, fluorescence spectroscopy and fluorescence polarization anisotropy. Results on a number of surfactants have agreed with those evaluated from conductometric, tensiometric and calorimetric methods. The absorbance and fluorescence intensities of curcumin are enhanced by different micellar solutions. The profiles of the absorbance and fluorescence intensities as a function of concentration of surfactant can be described by sigmoidal function to evaluate the cmc of the studied amphiphiles. However, we have found that some of the anisotropy profiles are sigmoidal in nature.

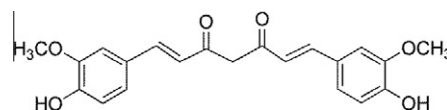
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1. Introduction

It is known that surfactants can assemble in solution in an ordered arrangement after reaching a specific concentration called critical micellar concentration (cmc) which is an important solution property of surfactants [1,2]. Almost all physical properties of a surfactant solution exhibit a sharp concentration dependent discontinuity in the region of self-aggregation or micellization. The cmc of a surfactant can be determined by various methods such as conductometry, tensiometry, fluorimetry, UV–VIS spectrophotometry, viscometry, calorimetry and NMR. Among these methods, spectroscopic methods are applied for the determination of cmc with the help of dye and other compounds as probes; self-absorption of surfactants in solution is also possible [3–6].

Curcumin is previously used as a probe for the determination of proteins in actual samples [9]. Here, first time, curcumin has been used as a probe for the determination of the cmc of various surfactants spectroscopically. Here, the values of cmc of various surfactants have been determined spectroscopically using curcumin as a dye and compared with the values obtained by other methods. Curcumin 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is the major ingredient of the naturally occurring yellow–orange pigments (curcuminoids) found in the Indian spice plant, turmeric and contains two ferulic acid molecules linked via a methylene bridge at the carbon atoms of the carboxyl groups.

Curcumin is a lipophilic molecule with phenolic groups and conjugated double bonds. This phenolic compound has wide range of physiological and medicinal effects [10]. The structure of curcumin is as follows:



Recently, curcumin has attracted much interest because several experimental studies have demonstrated that this natural polyphenol has anti-inflammatory [11], anti-oxidant [12], anti-Alzheimer's disease [13], anti-cystic fibrosis [14], antineoplastic, anti cancer and wound-healing effects [15], anti-angiogenic activities [16,17]. A major challenge in using curcumin for treatment of any disease is its poor aqueous solubility ($\sim 20 \mu\text{g/ml}$) which can be improved by increasing the pH of the solution. But this undergoes rapid degradation first by hydrolysis, followed by molecular fragmentation with the products being trans-6-(4'-hydroxy-3'-methoxy phenyl)-2,4-dioxo-5-hexenal, vanillin, ferulic acid and feruloyl methane [18]. Curcumin degradation can be diminished by encapsulating in surfactant micelles [19], thereby increasing its bioavailability prominently. However, the use of curcumin as a probe for the determination of cmc of surfactant has not been reported so far. In this paper, the study of the absorbance and fluorescence of curcumin

* Corresponding author. Fax: +91 33 24146266.

E-mail address: gsoumen70@hotmail.com (S. Ghosh).

has been performed in several micellar systems composed of cationic, anionic and nonionic amphiphiles to evaluate the cmc of amphiphiles as well as the exploration of the interaction of curcumin with the amphiphile.

To compare micellar cmc values obtained by the absorption and fluorescence methods, we have also used fluorescence polarization anisotropy to estimate cmc using curcumin as a fluorophore. The position of the fluorophore molecule in micellar aggregates can predict the number of oriented dipoles and can reflect the nature and changes of the surroundings of the fluorophore in surfactant assembly [20,21].

Since the cmc is the most important parameter to investigate the micellization of surfactants and in order to facilitate the experimental results obtained from different methods, the convenience of establishing curcumin as a standard probe to obtain the cmc from the absorbance or fluorescence intensity vs. concentration of surfactant plots is evident. All the experimental data obtained are analyzed and discussed in the context of determination of cmc.

1.1. Materials

Curcumin from *Curcuma longa* (Turmeric) was AR grade product of Sigma and methanol was a product of SRL (India). The anionic surfactants, sodium dodecyl benzene sulfonate (SDBS) and sodium *N*-dodecanoyl sarcosinate (SDDS) were purchased from Sigma and sodium dodecyl sulfate (SDS) from SRL (India). The cationic surfactants, dodecyltrimethylammonium bromide (DTAB) and tetradecyltrimethyl ammonium bromide (TTAB) were obtained from Sigma, cetyltrimethylammonium bromide (CTAB) from Alfa Aesar (UK) and octadecyltrimethyl ammonium bromide (OTAB) from Fluka. All nonionic surfactants, polyoxyethylene (20) sorbitan monolaurate (Tween-20), polyoxyethylene (20) sorbitan monopalmitate (Tween-40), polyoxyethylene (20) sorbitan monostearate (Tween-60) and polyoxyethylene (20) sorbitan monooleate (Tween-80) were purchased from Sigma (USA). All the surfactants were desiccated before use. Double distilled deionized water was used for all sample preparations and dilution.

1.2. Preparation of curcumin solution

A stock solution of curcumin (1 mg/ml in methanol) [19] was prepared by adding a known weight of the compound in methanol solution. The mixture was sonicated to yield a clear solution. A small quantity of this stock solution is dissolved in 2.5 ml water to achieve the experimental concentration of solution of curcumin where methanol concentration was very small. Such a small concentration of the methanol was considered not to affect the spectral and self-aggregation behavior of amphiphiles.

2. Experimental methods

2.1. UV–visible absorption studies

Absorbance measurements were performed in a UV 1601 Shimadzu (Japan) spectrophotometer using 10 mm path length quartz cuvette. The spectra were measured in 200–600 nm wavelength range. A small quantity of the curcumin stock solution (8.4 μ l) was added to 2.5 ml of water to achieve a final concentration of 9.1 μ M for curcumin. The concentrated surfactant solution was stepwise added into aqueous curcumin solution and absorption spectra were recorded after each addition.

2.2. Fluorescence emission and polarization anisotropy studies

The spectra and intensity of fluorescence emission and anisotropy using curcumin as the fluorescent probe were measured in

a Perkin Elmer LS 55 Fluorescence Spectrometer using a 10 mm path length quartz cuvette. A 50 μ l volume of the stock curcumin solution was added to 2.5 ml of water to achieve a concentration of 53.1 μ M for curcumin. Fluorescence spectra were recorded from 450 to 750 nm wavelength range with excitation and emission slit widths fixed at 10 nm. A concentrated surfactant solution was stepwise added into aqueous curcumin solution and the emission spectra were recorded after excitation. The scan time was fixed at 250 nm per minute. For polarization measurement, the wavelengths of excitation and emission were 423 nm and 553 nm respectively. The anisotropy value was taken as the average of five consecutive values. The sample temperature was allowed to stabilize at 300 K before each measurement.

3. Results and discussion

3.1. Absorption and emission spectra of curcumin

The absorption and emission spectra of curcumin in water are represented in insets of Fig. 1a and b. Curcumin has the absorption peak at 423 nm and the emission peak at 553 nm. Absorption spectra of curcumin in presence of various concentrations of Tween-40 and fluorescence spectra of curcumin in presence of various concentrations of Tween-80 are presented in Fig. 1a and 1b respectively. The absorption spectra have almost same peak position (423 nm) in different concentrations of surfactant (Tween-40). But fluorescence spectra in micellar solution are blue shifted from 553 nm to 527 nm at the lower concentration than cmc and it remains constant at 527 nm at and above cmc [22]. So the fluorescence

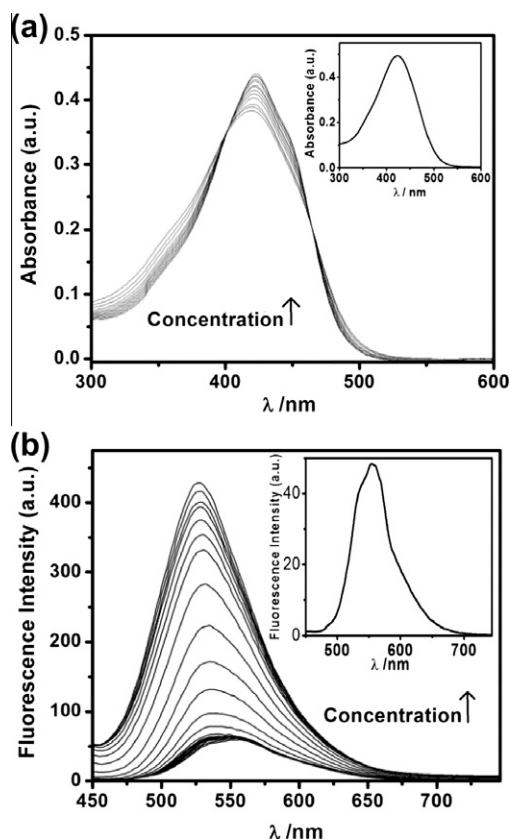
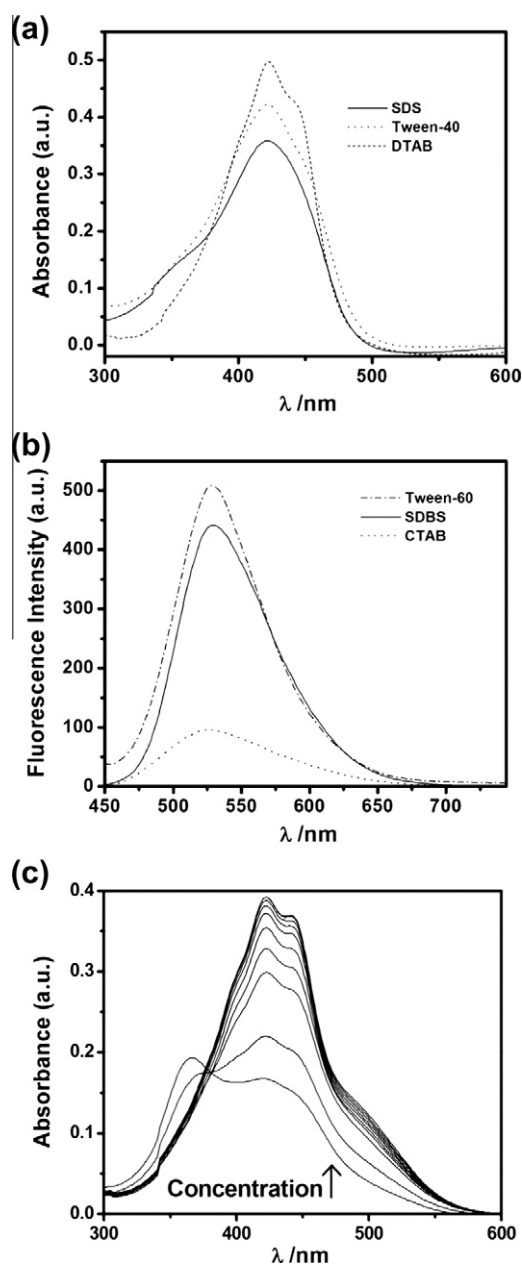
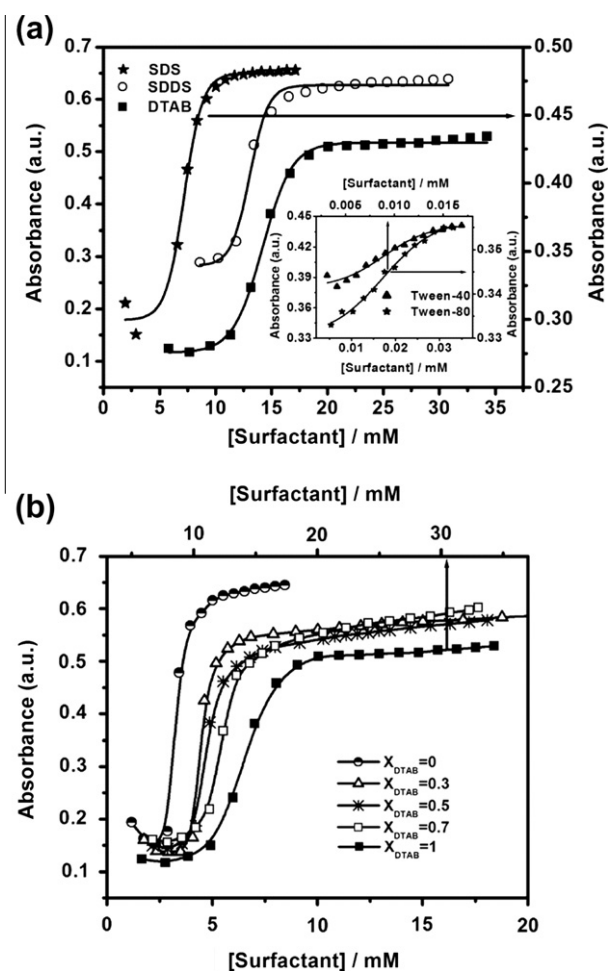


Fig. 1. (a) Absorption spectra of curcumin in various concentrations (0.00669–0.03282 mM) of Tween-40. Inset: absorption spectrum of curcumin in water at 300 K. (b) Fluorescence spectra of curcumin in various concentrations (0.00088–0.01856 mM) of Tween-80. Inset: fluorescence spectrum of curcumin in water at 300 K.

Table 1

The values of cmc of different surfactants obtained from different methods at 300 K.

Surfactants	cmc (mM)					
	Absorbance	Fluorescence	Anisotropy	Conductometry	Surface tension	Microcalorimetry
SDS	7.19	7.96	6.85	7.75 [4]	8.00 [4]	7.78 [4]
SDBS	0.86	1.21	–	1.18	1.00	–
SDDS	13.07	12.01	13.20	12.7 [25]	14.3 [25]	–
DTAB	14.2	13.88	–	15.30 [26]	14.6 [26]	15.2 [26]
TTAB	3.30	3.49	4.79	3.88 [26]	4.13 [26]	4.11 [26]
CTAB	0.76	0.75	–	0.91 [26]	0.82 [26]	0.97 [26]
OTAB	0.25	0.33	0.25	0.28 [27]	0.32 [27]	–
Tween-20	0.054	0.053	–	–	0.050 [4]	–
Tween-40	0.017	0.026	0.019	–	0.023 [5]	–
Tween-60	0.020	0.017	0.019	–	0.021	–
Tween-80	0.009	0.013	0.012	–	0.010 [6]	–

**Fig. 2.** (a) Absorption spectra of curcumin at cmc in SDS, Tween-40 and DTAB micellar solutions. (b) Fluorescence spectra of curcumin at cmc in Tween-60, SDBS and CTAB micellar solutions. (c) Absorption spectra of curcumin in various concentrations of CTAB at 300 K.**Fig. 3.** (a) Dependence of absorbance on surfactant concentration profile for SDS, SDDS and DTAB. Inset: absorbance vs [surfactant] profile for Tween-40 and Tween-80. (b) absorbance vs [surfactant] profile for different molar mixtures of mixed micelles having DTAB and TTAB at 300 K.

intensity has been measured at 527 nm for the determination of cmc of surfactant. The spectra in other micellar media are not illustrated to save space. The values of cmc are presented in Table 1 which shows the correlation of values obtained by different methods.

The absorption spectra at cmc in SDS, DTAB and Tween-40 micellar media are presented in Fig 2a. The spectrum for curcumin in DTAB micelle exhibits a structure with the presence of two peaks

Table 2

The values of cmc of binary mixtures of cationic surfactants DTAB and TTAB obtained from different methods at 300 K.

α_{DTAB}	cmc (mM)				
	Absorbance	Fluorescence	Conductometry [26]	Surface tension [26]	Microcalorimetry [26]
0.0	3.30	3.49	3.88	4.13	4.11
0.3	4.91	3.71	4.94	4.97	5.40
0.5	5.39	4.49	5.71	6.64	6.44
0.7	5.60	5.34	7.85	6.71	8.15
1.0	14.21	13.88	15.30	14.61	15.22

Table 3

The values of cmc of binary mixtures of cationic surfactants, CTAB and TTAB obtained from different methods at 300 K.

α_{CTAB}	cmc (mM)				
	Absorbance	Fluorescence	Conductometry [26]	Surface tension [26]	Microcalorimetry [26]
0.0	3.30	3.49	3.88	4.13	4.11
0.3	1.63	2.19	1.95	2.16	2.16
0.5	1.39	2.04	1.47	1.69	2.03
0.7	0.93	1.60	1.16	1.20	1.55
1.0	0.76	0.75	0.91	0.82	0.97

(approximately 423, and 445 nm); but this type of structure (excepting peak at 423 nm) is absent in SDS micelles and water [19]. The absence of vibronic structure in absorption band of curcumin indicates that it may interact strongly with water molecules in the Stern layer of the micelle. The absorption spectra are in the order: DTAB > Tween-40 > SDS for the same experimental condition. For SDBS, CTAB and Tween-60 amphiphiles the fluorescence spectra at their cmcs are presented in Fig. 2b. The emission peaks remain almost same in miellar media, but the intensity varies depending on the system. The spectrum of SDBS was intermediate between CTAB and Tween-60. Curcumin exhibits a broader spectrum peaked at 525 nm in CTAB micelle compared to that in other two micelles. This is probably due to binding of carbonyl groups of curcumin with cationic head group of CTAB.

Fig. 2c shows that curcumin in CTAB solution gives an absorption peak at 366 nm and a shoulder at 423 nm at lower concentration of CTAB. With increasing concentration of CTAB, the absorption peak at 366 nm loses its intensity and turns into shoulder whereas the peak at 423 nm increases gradually into a clear peak. At higher concentration of CTAB, the shoulder peak at 366 nm is disappeared and curcumin gives a pronounced absorption maximum at 423 nm with shoulder peak at 445 nm.

It is known that β -diketone moiety of curcumin can gradually chelate cationic metal ions. Thus positively charged head group of CTAB electrostatically interacts with the β -diketone group of curcumin to form CTAB-curcumin complexes. This binding decreases the extended aromatic conjugation of the planar geometry of curcumin [23]. As a consequence, curcumin gives absorption peak at 366 nm at lower concentration of CTAB. With increasing concentration, CTAB gradually leaves the β -diketone group of curcumin due to the strong hydrophobicity of aromatic groups of curcumin. Thus, conjugated structure of curcumin is recovered, and it gives the peak at 423 nm along with disappearing of peak at 366 nm [24]. DTAB and TTAB also show this type of spectra.

3.2. Absorption in surfactant solution

In Fig. 3a, plot of absorbance of curcumin against [surfactant] is plotted for SDS, SDDS and DTAB surfactants and inset shows that for Tween-40, and Tween-80. The profiles were all sigmoidal in nature and herein employed for cmc evaluation by fitting them to the Sigmoidal–Boltzmann equation (SBE) [8]. Thus,

$$A = \frac{a_i - a_f}{1 + e^{(x-x_0)/\Delta x}} + a_f$$

where the variable A corresponds to the absorbance value of curcumin, the independent variable (x) is the total concentration of surfactant, a_i and a_f are the initial and final asymptotes of the sigmoid respectively, x_0 is the center of the sigmoid and Δx is the parameter, which characterizes the steepness of the function. The sigmoidal plot produces cmc value at x_0 .

In addition to the depictions in Fig. 3a, illustrations for the sigmoidal absorbance vs [surfactant] plots are also represented for cationic mixed micelles having DTAB and TTAB surfactants at different mole fractions (Fig. 3b). It is known that mixed micelles have greater solubilization capacity than single micelle for the hydrophobic molecule indicating that cationic mixed micelles having DTAB and TTAB can provide more hydrophobic microenvironment for curcumin than single micelle resulting the enhancement of absorption of curcumin in mixed micelle. The cmc value of the system obtained by this method matches with our findings on the systems from conductance, surface tension and isothermal titration calorimetric measurements shown in Table 2. The profiles for the mixed micelles containing CTAB and TTAB have not been presented here to save space. The values of cmc of these systems have been depicted at different mole fractions (α) of CTAB in Table 3. Fig. 4 shows the plot of absorbance peak of curcumin against [surfactant] for Tween-20 and Tween-60. The cmc value has been determined

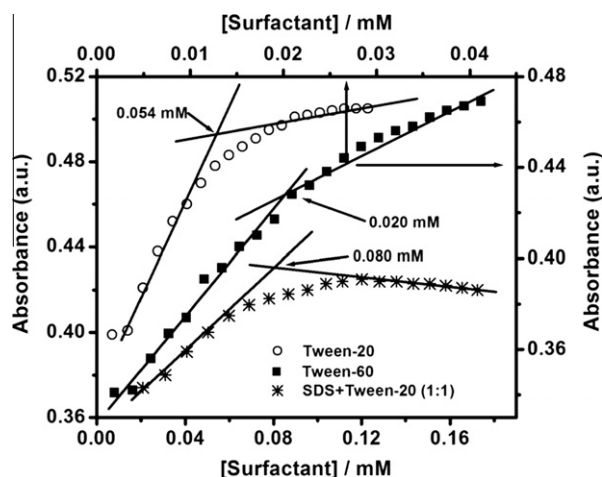
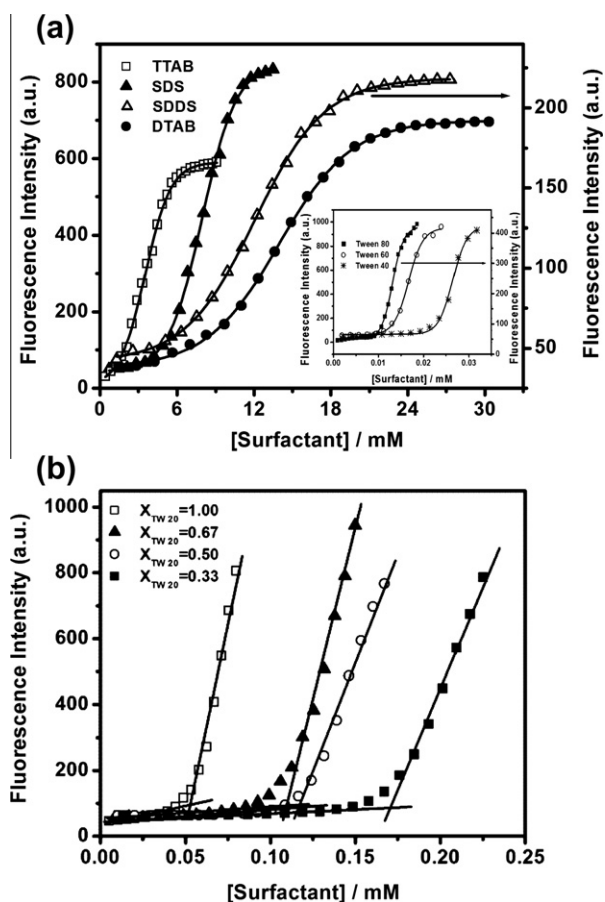


Fig. 4. Effect of absorbance of curcumin by different concentrations of surfactants for Tween-20, Tween-60, and equimolar mixture of SDS and Tween-20 at 300 K.

Table 4

The values of cmc of binary mixtures of Tween-20 and SDS obtained from different methods at 300 K.

$\alpha_{\text{Tween-20}}$	cmc (mM)			
	Absorbance	Fluorescence	Conductometry [4]	Surface tension [4]
0.00	7.19	7.96	7.75	8.00
0.33	0.10	0.17	0.13	0.13
0.50	0.08	0.12	0.09	0.09
0.67	0.06	0.11	0.07	0.07
1.00	0.05	0.05	–	0.05

**Fig. 5.** (a) Dependence of fluorescence intensity on concentration of surfactant for TTAB, SDS, SDDS and DTAB. Inset: fluorescence intensity vs [surfactant] profile for Tween-80, Tween-60 and Tween-40. (b) Effect of fluorescence intensity of curcumin by different molar mixtures of mixed micelles containing Tween-20 and SDS at 300 K.

from the break point of premicellar and postmicellar regions. The premicellar region has greater slope than that of postmicellar one. These cmc values have been mentioned in Table 1. Here if we try to determine cmc according to Sigmoidal–Boltzmann equation, we can get x_0 value which is widely different from actual value. Hence we determine cmc by applying tangential fit, which

matches nicely with the literature value, obtained by surface tension, microcalorimetric methods, etc. [8]. In the case of SDS and Tween-20 mixtures, we get the cmc by the similar type of break point, but interestingly, premicellar slope is positive and postmicellar slope is negative. After cmc, due to the interaction between anionic head group of SDS and polyoxyethylene group of Tween-20, curcumin cannot enter into the hydrophobic core of the micelle, so absorbance decreases. The cmc value has been shown in Table 4.

3.3. Emission in surfactant solution

In Fig. 5a, the emission intensities of curcumin against [surfactant] for TTAB, SDS, SDDS, DTAB and in inset Tween-40, Tween-60 and Tween-80 are plotted. The profiles are all sigmoidal in nature. So using Sigmoidal–Boltzmann equation, the cmc values have been evaluated and presented in Table 1. Fig. 5a shows that fluorescence intensity is the lowest for SDDS among ionic surfactants and Tween-80 among nonionic surfactants. Fig. 5b shows the emission intensities of curcumin against [surfactant] for Tween-20 and SDS mixtures. Here, the cmc value has been determined from the intersecting point of two tangents drawn on premicellar and postmicellar regions. Below the cmc, fluorescence intensity is nearly independent of concentration of surfactant and above the cmc, it increases very rapidly due to greater solubilization of curcumin resulting in the formation of ion association complex by electrostatic force in more hydrophobic microenvironment of the mixed micelle of SDS/Tween-20 systems. This kind of plot is obtained in case of OTAB which has not been shown to save space. The values of cmc of different binary mixtures are presented in Table 4 and compared with cmc obtained by other methods [4].

Similarly, the cmc values of binary mixtures of anionic combinations of SDS and SDBS have been exemplified by absorbance and fluorescence methods at different mole fractions of SDS (α_{SDS}) in Table 5. Such cmc values of the mixtures are lower compared to those obtained by other methods [28].

3.4. Fluorescence polarization anisotropy in surfactant solution

The fluorescence anisotropy (r) is defined as:

$$r = \frac{(I_v - GI_h)}{(I_v + 2GI_h)}$$

where I_v and I_h are the respective fluorescence intensities of the vertically and horizontally polarized emission when the sample is

Table 5

The values of cmc of binary mixtures of SDS and SDBS obtained from different methods at 300 K.

α_{SDS}	cmc (mM)				
	Absorbance	Fluorescence	Conductometry	Surface tension	Microcalorimetry [28]
0.0	0.86	1.21	1.18	1.00	1.80
0.3	1.31	1.52	2.60 [28]	2.22 [28]	2.68
0.5	1.75	1.78	3.60 [28]	3.57 [28]	3.32
0.7	1.94	2.19	5.07 [28]	4.02 [28]	5.10
1.0	7.19	7.96	7.75 [4]	8.00 [4]	7.78

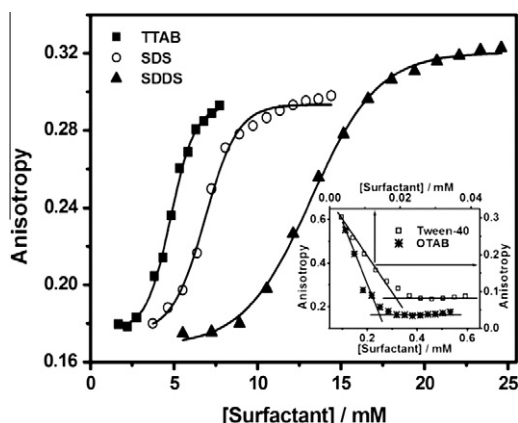


Fig. 6. Dependence of fluorescence anisotropy on concentration of surfactant for TTAB, SDS and SDDS. Inset: fluorescence anisotropy vs [surfactant] profile for Tween-40 and OTAB at 300 K.

excited with vertically polarized light. The *G* factor denotes the ratio of the sensitivities of the detection system for vertically and horizontally polarized light [21].

Anisotropy should show a significant difference (0.1 or more) from the base line value (i.e. in aqueous solution with no surfactant added) at higher ranges of surfactant concentration to obtain reliable results [20]. Curcumin has satisfied this condition for the determination of cmc of surfactant. Fig. 6 shows the dependence of fluorescence anisotropy on different concentrations of surfactants for TTAB, SDS and SDDS. The profiles are all sigmoidal in nature; sudden change in anisotropy produces cmc value which is evaluated using Sigmoidal–Boltzmann equation. Here, anisotropy value increases with increasing surfactant concentration. This reflects a change in the environment of the curcumin. With increasing concentration of surfactant, curcumin can bind with surfactant molecules having a tendency towards rotational movement of curcumin resulting in high anisotropy values. At cmc, curcumin becomes solubilized within the hydrophobic core of the micelle. In case of Tween-40, Tween-60, and OTAB, anisotropy decreases with increasing surfactant concentration below the cmc and leveled off above the cmc. These surfactants have very low cmc values compared to other surfactants. Probably, with increasing concentration of amphiphile, the binding tendency of curcumin with amphiphile is less leading to decrease in anisotropy [7]. At cmc, curcumin becomes solubilized possessing lower microviscosity. But, curcumin cannot determine the cmc of DTAB, CTAB, SDBS and Tween-20 indicating non-suitability of the fluorophore, curcumin as a probe towards these surfactants for the determination of cmc. It is because the photophysical properties are shown to be sensitive to their local environment. Anisotropy is dependent on the proper orientation of the fluorophore. In DTAB, CTAB, SDBS and Tween-20 solutions, fluorophore, curcumin takes those orientations which are not sensing the morphological changes in the micellar aggregates. Thus, we cannot measure cmc of every surfactant solution by anisotropic measurement using a single probe which is not uncommon and reported earlier [20].

4. Conclusions

In this work, it is found that single and mixture of surfactants can significantly enhance the intensity of both absorbance and fluorescence spectra of curcumin. These measurements suggest a role for hydrophobic interaction between curcumin and micellar system. The absorption and emission results of curcumin in different surfactant solutions have evidenced fair agreement towards their cmc values with those obtained by other methods. So these

methods can be convenient techniques for the determination of cmc of a surfactant using an antioxidant. Curcumin can be used as a fluorophore in fluorescence polarization anisotropy measurement to determine cmc of surfactant and to study the interaction between them.

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