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Spectroscopic Study on the Interaction of Medicinal Pigment, Curcumin with Various Surfactants : An Overview

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Abstract — Curcumin ($C_{21}H_{20}O_6$), a natural antioxidant is the active ingredient of turmeric powder and is well documented for centuries for its medicinal properties owing to the treatment of a variety of inflammatory conditions and other diseases in Indian and Chinese systems of medicine. Curcumin is poorly water soluble. Recently, different micellar systems have been used for the incorporation of curcumin over a wide range of pH values to investigate its solubility and stability with the help of spectroscopic techniques. Curcumin is also used as a probe for the determination of the critical micellar concentrations of different amphiphiles spectroscopically. This pigment behaves in a different way depending on the type of the micelles and pH of the medium. This review summarizes all such investigations.

Keywords : *Curcumin, Surfactants, UV-VIS absorption, Fluorescence, Polarization anisotropy.*

INTRODUCTION

Curcumin 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a natural antioxidant (strong activity compared to vitamins C and E) 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 [1-4]. Curcumin or diferuloylmethane is a lipophilic molecule containing phenolic groups and conjugated double bonds. Curcumin exists in two forms : one is keto-enol form and the other is diketo one (Fig. 1). In solution, it exists predominately in enol form. Curcumin

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possesses a variety of biological and pharmacological activities. It is used extensively in Ayurvedic, Unani and Siddha medicines for different diseases [5]. Recently, curcumin has attracted much interest to researchers because several experimental studies show that this natural polyphenol has anti-inflammatory [6], anti-oxidant [7], anti-Alzheimer's disease [8], anti-cystic fibrosis [9], antineoplastic, anti cancer and wound-healing effects [10], anti-angiogenic activities [11]. Oral take up of curcumin with minimal acute has the great value to overcome these chronic illnesses [10]. Major problem with curcumin for the treatment of diseases is its reduced bioavailability i.e., its poor solubility in water at acidic and neutral pHs making hard to absorb and also the lack of stability in aqueous medium. Curcumin is stable at acidic pH but is degraded in alkaline medium to trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane, and vanillin [12]. Such degradation can be reduced through encapsulation in micellar media and increasing the bioavailability of curcumin prominently [1].

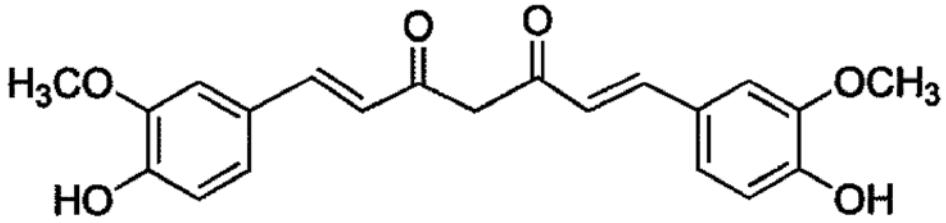

Fig. 1. (a) Structure of curcumin in keto form

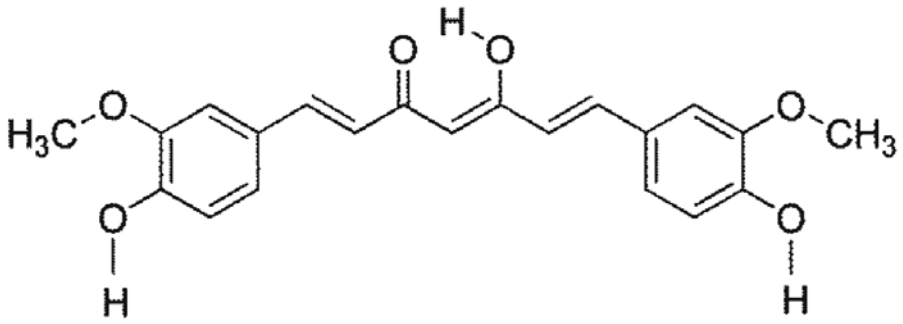

Fig. 1. (b) Structure of curcumin in enol form

Many synthesized and natural drugs have poor aqueous solubility. Surfactants are widely used in drug industry to enhance aqueous solubility, drug stability and

to control drug release and uptake. Surfactants are used to increase the bioavailability of drugs. Therefore, the interaction between surfactant and drug has an important value. Colloidal solution of surfactant which forms micelles and vesicles are commonly used for solubilising and stabilising hydrophobic molecules which are sparingly soluble in aqueous solutions. Hydrophobic curcumin is easily solubilized in micellar solutions upto about 40 times [13]. At pH 5 and 8, sodium dodecyl sulphate (SDS), Triton X-100 (TX-100), and tetradecyl trimethylammonium bromide (TTAB) micelles are highly effective in stabilizing curcumin by nearly 1800 times [14]. Curcumin has the free radical scavenging ability in neutral and cationic micellar solutions [15]. Recently, it is found that binding of curcumin with phospholipid micelles can significantly change the micro-structural properties of micelles and curcumin molecules are incorporated into the phospholipid bilayer in a transbilayer orientation anchored by hydrogen bonding to the phosphate group of lipids and hydrophobic interaction [16]. Although, curcumin has wide range of physiological and medicinal effects, there are still a limited number of investigations on curcumin in micellar systems which may provide valuable insight into the properties of curcumin in biomembranes. In this review article, we discuss thoroughly the interaction of curcumin with various types of surfactants using spectroscopic methods (UV-VIS absorption, steady state fluorescence and fluorescence polarization anisotropy).

UV-VIS ABSORPTION SPECTRA OF CURCUMIN IN PRESENCE OF SURFACTANTS

Curcumin has an intense optical absorption peak in the UV-visible spectral region in water and CTAB micellar solutions at 423 nm and the values of molar extinction coefficient ranging from 25 000 to 60 000 M^{-1} cm^{-1}, presenting in Fig. 2 [1]. The UV-VIS absorption spectra from Fig. 3 represent the plots of curcumin in different micellar solutions at their individual critical micellar concentration (cmc) values. These plots show a structure of curcumin in presence of cationic micelle at 423 and 445 nm; but this structure is absent in SDS micelles and water [4].

Detail investigation regarding absorbance of curcumin in CTAB solution exhibits an absorbance peak at 366 nm and a shoulder at 423 nm at lower concentration of surfactant [Fig. 4]. Actually, positively charged head group of CTAB binds with the β-diketone group of curcumin through electrostatic interaction to form CTAB-curcumin complexes [4]. Such binding helps to decrease the extended aromatic conjugation of the planar geometry of curcumin. So absorption peak appears at 366 nm at lower concentration of CTAB. At higher concentration, CTAB leaves the β-diketone group

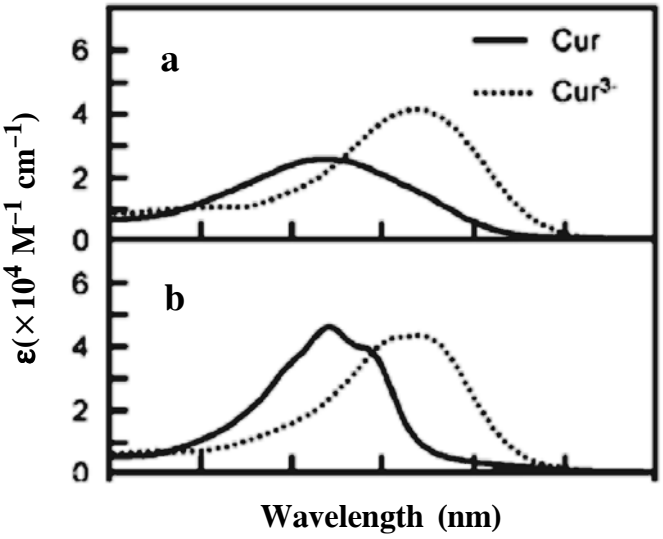

Fig. 2. UV-VIS absorption spectra of curcumin (50 μM) and Cur^{3-} in (a) water, and (b) CTAB [1] (with permission from ACS).

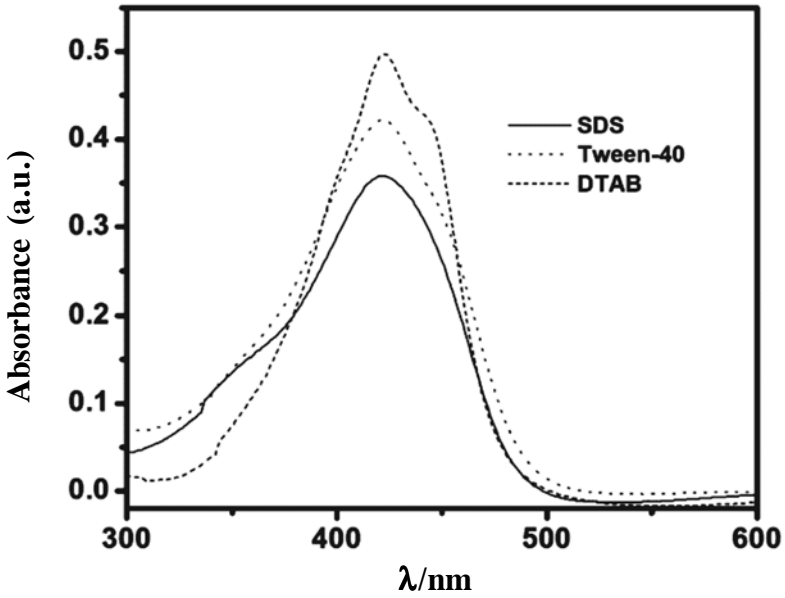

Fig. 3. Absorbance spectra of curcumin (9.1 μM) at cmc in SDS, Tween-40 and DTAB micellar solutions [4] (with permission from Elsevier).

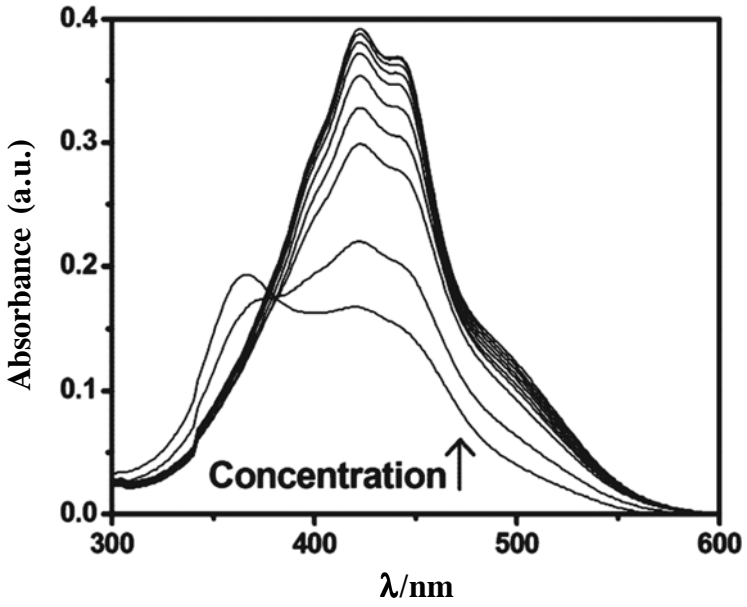

Fig. 4. Absorbance spectra of curcumin in presence of different concentrations of CTAB at 300 K [4] (with permission from Elsevier).

of curcumin due to the strong hydrophobic nature of aromatic groups of curcumin recovering its conjugated structure and peak appears at 423 nm [4]. Again, the plot of absorbance of curcumin against various concentrations of surfactants, such as SDS, DTAB, sodium N-dodecanoyl sarcosinate (SDDS), polyoxyethylene (20) sorbitan monopalmitate (Tween-40), and polyoxyethylene (20) sorbitan monooleate (Tween-80) show the sigmoidal curves in nature in Fig. 5 where cmc values of those surfactants have been determined by fitting the plots to the Sigmoidal-Boltzmann equation and presented in Table 1 [4].

Mixed micelles having DTAB-TTAB, CTAB-TTAB as well as SDS-SDBS follow the same trend and the first two combinations have been shown in Fig. 5. The cmc values have been presented in Tables 2 and 3 which also correlate with the values determined by other methods [4, 17-23]. In case of polyoxyethylene (20) sorbitan monolaurate (Tween-20), polyoxyethylene (20) sorbitan monostearate (Tween-60), and SDS-Tween-20 mixtures, the cmc value (Table 3) has been evaluated from the break point of premicellar and post micellar regions by applying tangential fit [4]. The spectrometric titration of curcumin in cationic and anionic micelles against

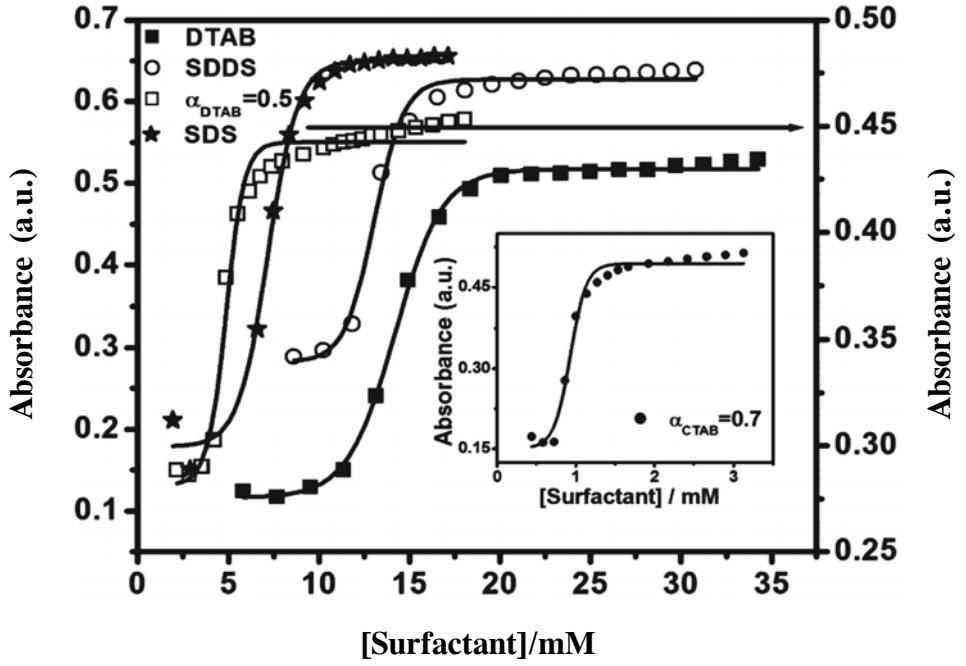

Fig. 5. Dependence of absorbance on surfactant concentration profile for SDS, SDDS, DTAB and equimolar mixture of mixed micelle having DTAB and TTAB at 300K. Inset shows absorbance vs. [surfactant] profile for (7 : 3) molar mixture of mixed micelle having CTAB and TTAB at 300K [4] (with permission from Elsevier).

different pH show that the spectra of curcumin undergo complex change without isosbestic points [2]. Each plot shows a sigmoidal transition with increasing pH of the solution. Such observation indicates the strong interaction between curcumin and cationic (CTA^+ or DTA^+) or anionic (DBS^- of SDBS) micellar system [2].

In alkaline medium, curcumin has a tendency to degrade rapidly to form trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal as the main product with further degradation to vanillin, ferulic acid and feruloyl methane [12]. English et al. [2] showed the absorption spectra of curcumin in cetyl trimethyl ammonium tosylate (CTAT) and sodium dodecylbenzene sulfonate (SDBS) micellar media at pH 9.2 in Fig. 6. Here, in 4 mM SDBS, the rate of degradation of curcumin is lower than than the rate in water indicating a significant interaction between curcumin and SDBS micelles [2]. In case of CTAT micelles, curcumin behave differently at pH 9.2. The

TABLE 1.

The values of cmc of different surfactants obtained by spectroscopic methods at 300 K [4] (with permission from Elsevier).

Surfactants	cmc (mM)		
	Absorbance	Fluorescence	Anisotropy
SDS	7.19	7.96	6.85
SDBS	0.86	1.21	–
SDDS	13.07	12.01	13.20
DTAB	14.2	13.88	–
TTAB	3.30	3.49	4.79
CTAB	0.76	0.75	–
OTAB	0.25	0.33	0.25
Tween-20	0.054	0.053	–
Tween-40	0.017	0.026	0.019
Tween-60	0.020	0.017	0.019
Tween-80	0.009	0.013	0.012

TABLE 2.

The values of cmc of binary mixtures of TTAB-DTAB and TTAB-CTAB obtained from absorbance and fluorescence methods at 300 K [4] (with permission from Elsevier).

α_{TTAB}	cmc (mM)			
	TTAB and DTAB		TTAB and CTAB	
	Absorbance	Fluorescence	Absorbance	Fluorescence
0.0	14.21	13.88	0.76	0.75
0.3	5.60	5.34	0.93	1.60
0.5	5.39	4.49	1.39	2.04
0.7	4.91	5.34	1.63	2.19
1.0	3.30	3.49	3.30	3.49

spectrum in presence of CTAT micelles shows a strong red shift in the absorption spectrum and the degradation process is stopped denoting the partitioning of curcumin

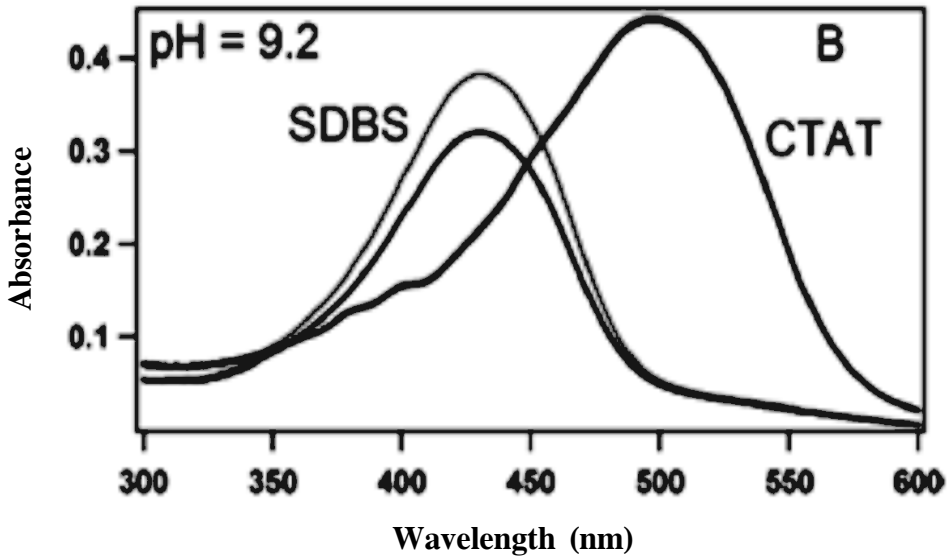

Fig. 6. Spectroscopic measurements of 9.1 μM curcumin at pH 9.2 in 4 mM SDBS and CTAT micelle. For SDBS, light curve spectra were taken of the fresh sample and 1 hour later (deep curve) [2] (with permission from ACS).

TABLE 3.

The values of cmc of binary mixtures of SDS-SDBS and SDS-Tween-20 obtained from absorbance and fluorescence methods at 300 K [4] (with permission from Elsevier).

α_{SDS}	cmc (mM)			
	SDS and SDBS		SDS and Tween-20	
	Absorbance	Fluorescence	Absorbance	Fluorescence
0.0	0.86	1.21	0.05	0.05
0.3	1.31	1.52	0.06	0.11
0.5	1.75	1.78	0.08	0.12
0.7	1.94	2.19	0.10	0.17
1.0	7.19	7.96	7.19	7.96

to the cationic micellar phase with blocking the reaction pathway [2]. Similar observation is made for curcumin in other cationic micelles. Such type of red shift denotes that there is a significant interaction between curcumin and CTA^+ ion

stabilizing the phenoxide ions through electrostatic and hydrophobic interactions. Significant red shift is also observed in aqueous buffer only at pH > 10. In this region, Cur^{2-} and Cur^{3-} species are formed and result in an increase in red shifted absorption band [2]. In the kinetics of degradation of Cur^{3-} (pH = 13), the absorbance maximum degrades to roughly 67% of the original value in 20 h in aqueous solution [1]. In cationic micellar solution, this degradation is minimum. Actually, cationic micelles stabilize Cur^{3-} and suppress degradation [1]. In the anionic micellar solution having SDS, the rate of degradation is high similar to that in aqueous solution denoting the ineffective nature of SDS to reduce the rate of degradation.

Again, Tønnesen [14] reported that in a weakly alkaline solution (at pH 8), curcumin undergoes rapid degradation in CTAB micelles where as, SDS micelles strongly prevent the degradation of curcumin. The difference between such results at pH 8 and 13 denotes that the degradation pathway is highly pH sensitive resulting the rate of deprotonation of curcumin in case of cationic micelles [1]. Generally, one third of curcumin molecules are deprotonated at pH 8 following the Henderson-Hasselbalch equation whereas at pH 13, curcumin is fully deprotonated [1]. In case of anionic micelle (particularly SDS), the inhibition nature for the degradation of curcumin at pH 8 is due to the electrostatic repulsion between the OH^- ions and the negatively charged surfactant head group resulting the segregation of curcumin from the OH^- ions. But at pH 13, Cur^{3-} is formed, which rapidly degrades in SDS micelles signifying the dissociation of Cur^{3-} from the micelle to the aqueous phase. Wang et al [24] reported that the absorbance of curcumin in mixed micelle of CTAB and SDBS is higher than that in individual micelle with the formation of ion association following the solubilization of curcumin in mixed micelle. They also evaluated the fluorescence quantum yield of curcumin in the mixed micelle that is 55 times higher than that in aqueous solution having 1% ethanol. Absorption spectra of curcumin in different concentrations of non-ionic surfactant, tert-octyl phenyl polyoxyethylene ether (Triton X-100 or TX-100) show that absorbance decreases with increasing concentration of TX-100 ranging from 0.02–0.1 mM and increases at and above cmc (0.2 mM) [25]. Here, association rate constant of curcumin in TX-100 micelle is in between those of CTAB and SDS micelles where CTAB is bound to curcumin with the highest affinity. This may be due to the electrostatic interaction between curcumin (deprotonated anionic form) and positively charged head group of CTAB whereas the repulsion between deprotonated enol (anionic) of curcumin and negatively charged head group of SDS is responsible for a weaker interaction following to decrease the rate constant [25].

STEADY-STATE FLUORESCENCE SPECTRA OF CURCUMIN IN PRESENCE OF SURFACTANT

Curcumin emits relatively weak fluorescence with the emission peak at 553 nm in aqueous medium. The fluorescence spectra of curcumin in presence of different concentration of Tween-80 are blue shifted from 553 nm to 527 nm at the lower concentration than cmc and these remain constant at 527 nm at and above cmc (Fig. 7) [4]. The fluorescence intensities of curcumin against concentration of surfactant have been depicted in Fig. 8 for different surfactants. All curves are sigmoidal in nature and the cmc values of surfactants have been determined using Sigmoidal-Boltzmann equation presenting in Table 1.

Fig. 8 shows the lowest fluorescence intensity of SDDS among ionic surfactants and Tween-80 among nonionic amphiphiles [4]. For the binary combinations of SDS and SDBS, cmc values have been determined from sigmoidal curves obtained (not

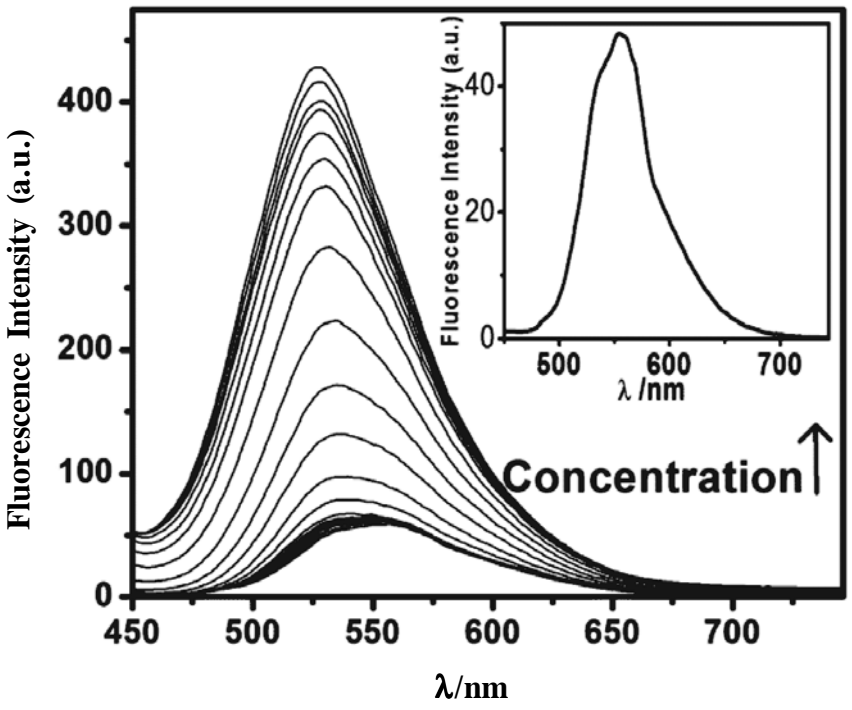

Fig. 7. Fluorescence spectra of curcumin in different concentrations (0.00088–0.01856 mM) of Tween-80. Inset : Fluorescence spectrum of curcumin in water at 300K [4] (with permission from Elsevier).

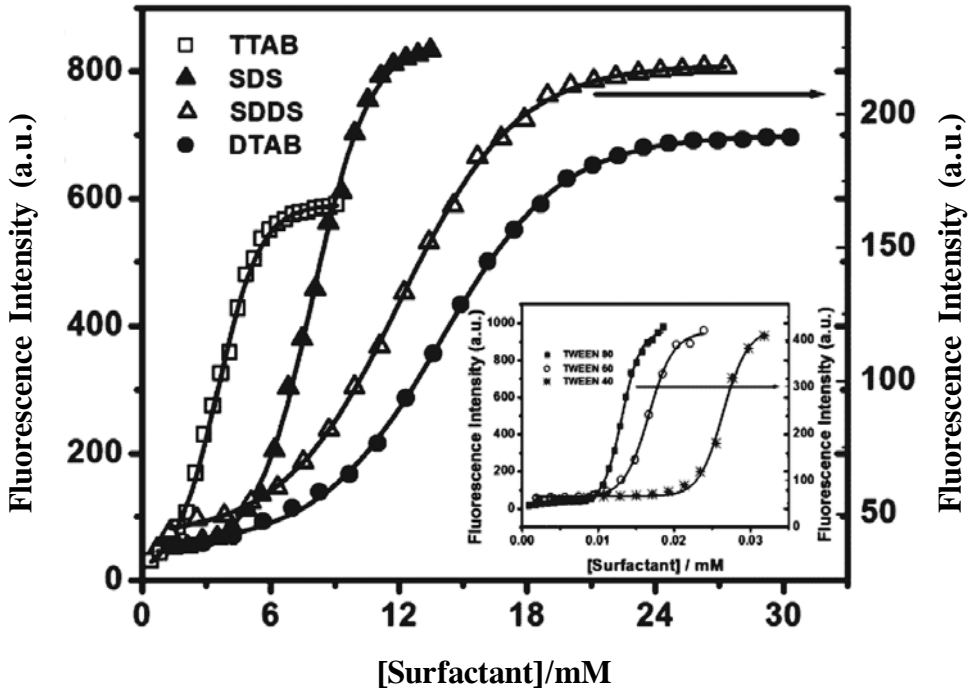

Fig. 8. Fluorescence intensity vs. [surfactant] profile for TTAB, SDS, SDDS and DTAB. Inset shows same type of profile for Tween-80, Tween-60, Tween-40 [4] (with permission from Elsevier).

shown to save space) and presented in Table 3. In case of Tween-20 and SDS mixtures, the cmc values have been evaluated from the break point of two tangents drawn on premicellar and postmicellar areas and exemplified in Table 3. For such system, intensity is initially independent of concentration of surfactant and above the cmc, very rapid increase in intensity is observed owing to higher solubilization of curcumin following the formation of ion-association complex through electrostatic force in higher hydrophobic microenvironment of the mixed micellar systems. Octadecyl trimethyl ammonium bromide (OTAB) also shows similar type of curve. These plots have been presented in Fig. 9.

Shen et al. [3] reported the fluorescence spectra of curcumin in presence of different concentrations of DTAB at pH 5. Under this condition, curcumin shows the emission peak at 570 nm in absence of surfactant. The fluorescence intensity of curcumin decreases in presence of DTAB having one-third concentration of cmc or

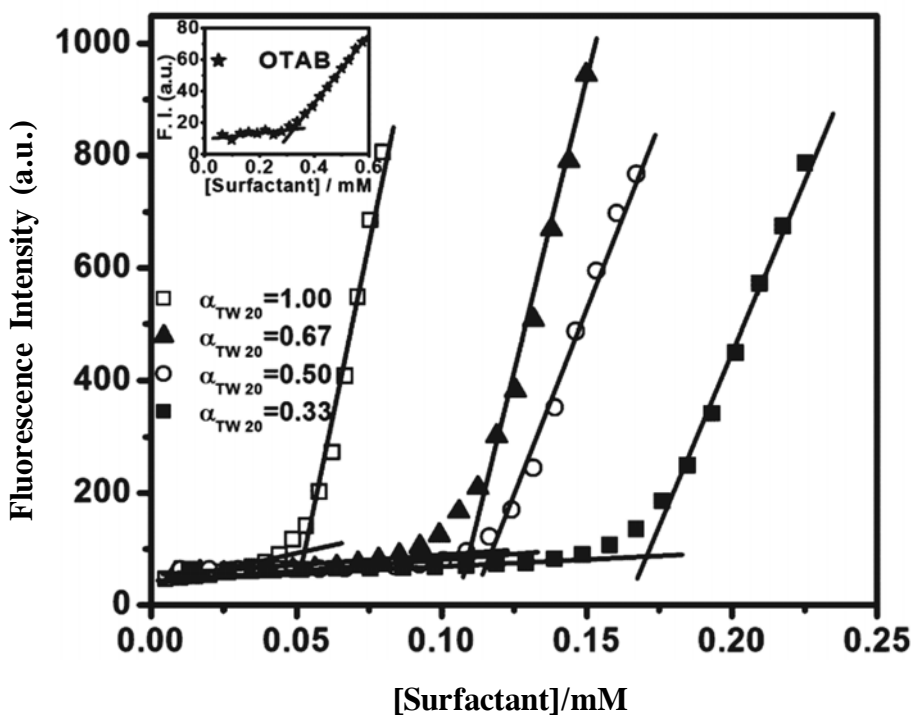

Fig. 9. Dependence of fluorescence intensity of curcumin for different molar mixtures of mixed micelles having Tween-20 and SDS at 300 K [4] (with permission from Elsevier). Inset shows the plot of OTAB.

less owing to the formation of DTAB/curcumin complexes and partly due to the quenching effect of the Br^- of DTAB. But at and around cmc, there is an increase in the fluorescence intensity with a blue shift compared to that of curcumin without surfactant due to lower polarity of DTAB premicelles or micelles (Fig. 10). At very high concentration of DTAB, the high fluorescence intensity denotes that the formed aggregates can greatly enhance the intensity of curcumin because of the nonpolar environment of the system and no quenching effect of the Br^- ions [3].

Results of the fluorescence measurements of Cur^{3-} in aqueous and micellar solutions show that the fluorescence intensities of Cur^{3-} in CTAB and DTAB micelles are almost ten times higher than that in aqueous as well as SDS solution signifying the encapsulation of Cur^{3-} in those cationic micelles preventing degradation [1]. Similarly, identical fluorescence spectra of Cur^{3-} in the SDS and aqueous solution denote that Cur^{3-} is dissociated from the SDS micelles liberating to the aqueous phase.

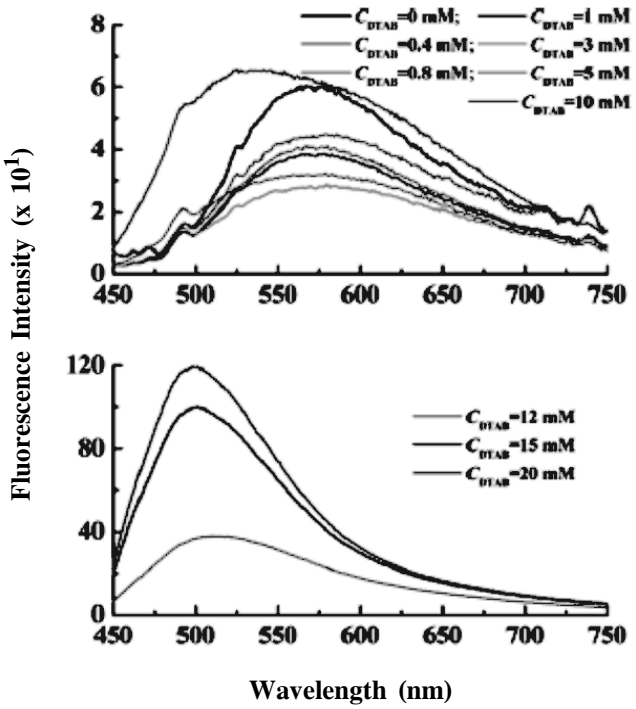

Fig. 10. Steady-state fluorescence spectra of curcumin in presence of different concentrations of DTAB in sodium phosphate buffer medium (pH = 5) at 298K [3] (with permission from ACS).

Again, the fluorescence intensity can be highly enhanced almost 55 times by the mixed micelle of SDBS and CTAB using HOAc-NaOAc buffer for adjusting pH = 4 compared to that of curcumin in aqueous medium and the peak has a blue shift to 494 nm denoting the solubilization of curcumin in mixed micelle [24]. It is also reported that the fluorescence intensity of curcumin in various concentrations of TX-100 increases with increasing concentration of surfactant [25].

FLUORESCENCE POLARIZATION ANISOTROPY

Fluorescence polarization technique provides the information on the anisotropy (r) and the microviscosity (η) of the environments of curcumin. The fluorescence anisotropy (r) can be 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 polarised emission when the sample is excited with vertically polarised light. The factor, G is the ratio of the sensitivities of the detection system for vertically and horizontally polarized light [4].

Study of fluorescence anisotropy of curcumin on various concentrations of surfactants has been done and presented in Fig. 11 [4]. The curves are sigmoidal in nature for TTAB, SDS and SDDS where sudden change in anisotropy gives the cmc value calculating from Sigmoidal-Boltzmann equation. With increasing concentration of surfactant, anisotropy value increases denoting the binding of curcumin with surfactant molecules and solubilization of curcumin within the hydrophobic core of the micelle. But in case of Tween-40, Tween-60 and OTAB, decrease in anisotropy

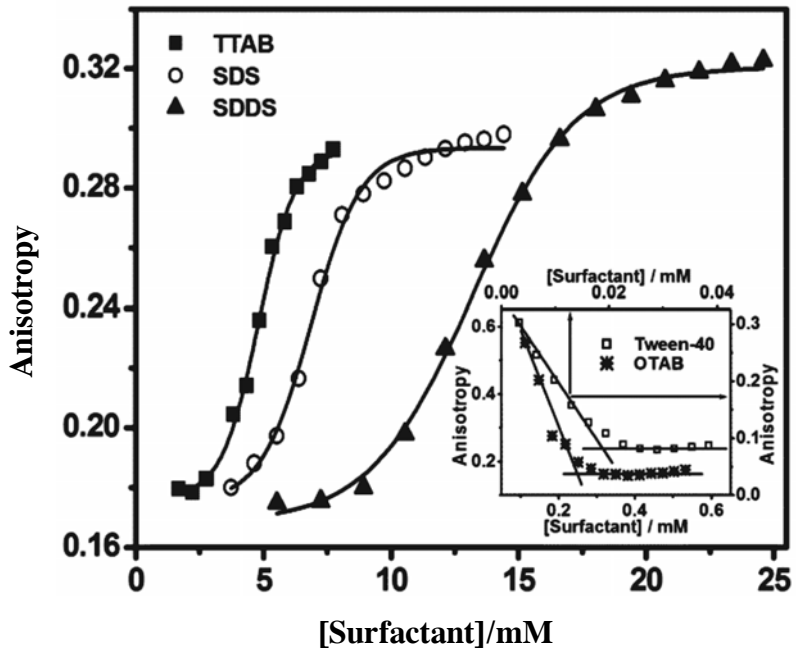

Fig. 11. Dependence of fluorescence anisotropy on concentration of surfactant for TTAB, SDS and SDDS. Inset: Fluorescence anisotropy vs. [Surfactant] profile for Tween-40 and OTAB at 300K [4] (with permission from Elsevier).

with increasing concentration of amphiphile is observed below the cmc and constant above the cmc indicating less binding tendency of curcumin with surfactant [4]. Again, the cmc of DTAB, CTAB, SDBS and Tween-20 cannot be determined using curcumin because of non-suitability of the fluorophore, curcumin as a probe towards those surfactants. Actually, in those surfactant solutions, curcumin does not take suitable orientations to sense the morphological changes in the micellar aggregates [4]. Microviscosity (η) of the system can be evaluated from the following equation [3],

$$\eta = 2.4r / (0.362 - r)$$

In Fig. 12, the microviscosity values have been plotted against concentration of surfactant. Like anisotropic values, the studied amphiphiles follow almost the same trend in case of microviscosity.

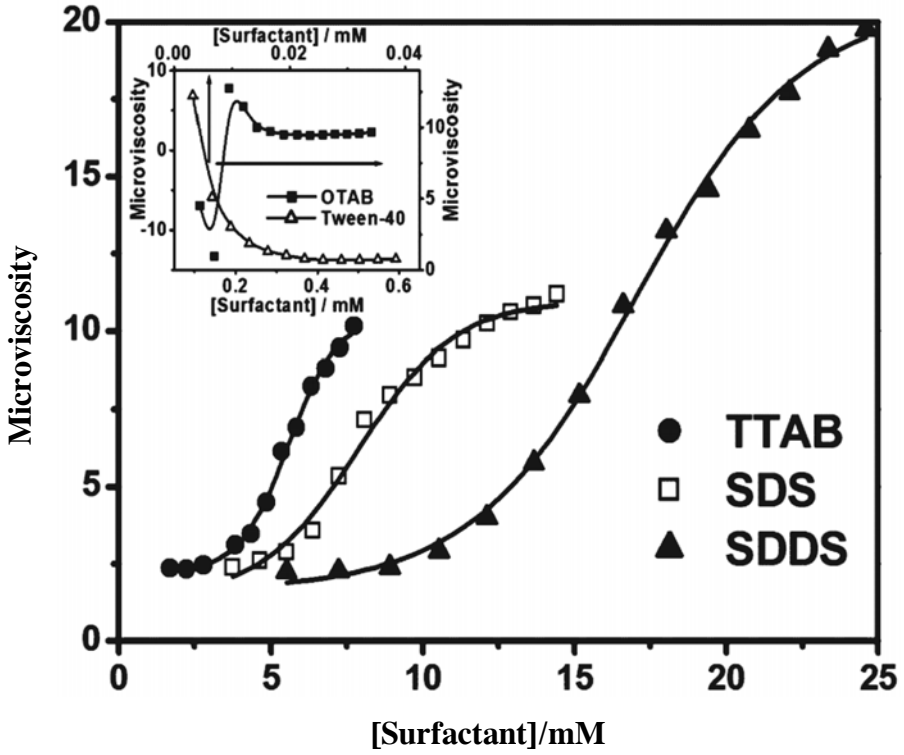

Fig. 12. Microviscosity vs. [Surfactant] for TTAB, SDS and SDDS. Inset shows dependence of microviscosity on concentration of surfactant for Tween-40 and OTAB at 300K.

Variations of the anisotropy and the microviscosity of curcumin have been done in DTAB solution using sodium phosphate buffer at pH = 5 [3]. At the lower range of concentration (0–10 mM) of DTAB, anisotropy decreases with increasing concentration of surfactant, but microviscosity is almost same denoting monomeric form of DTAB. At and above cmc of DTAB, ascending trend of both values is observed, signifying higher restriction of the rotation of curcumin molecule as well as aggregation of the amphiphile [3].

CONCLUSION

Recent developments on the interaction between curcumin and different types of surfactants at various pH values have been reviewed which show that single and mixture of amphiphiles can remarkably enhance the intensity of both absorbance and fluorescence spectra of curcumin. Here, electrostatic and hydrophobic interactions between curcumin and micellar systems play a major role. Cationic surfactants are more effective than anionic one to stabilize this pigment owing to its synergistic electrostatic interactions with the cationic head groups against alkaline hydrolysis. Such type of interaction can give the idea for the further improvement of a water soluble and stable curcumin product which is necessary for its potential pharmaceutical applications.

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ERRATA (added after completion of Printing)

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The Corrected legends of Fig. 2, 6 and 10 are the following :

Fig. 2. UV-VIS absorption spectra of curcumin (50 μM) and Cur^{3-} in (a) water, and (b) CTAB [1]; Reprinted (adapted) with permission from (M. H. M. Leung, H. Colangelo, T. W. Kee, Langmuir, 24, 5672 (2008)). Copyright (2008) American Chemical Society.

Fig. 6. Spectroscopic measurements of 9.1 μM curcumin at pH 9.2 in 4 mM SDBS and CTAT micelle. For SDBS, light curve spectra were taken of the fresh sample and 1 hour later (deep curve) [2]; Reprinted (adapted) with permission from (Z. Wang, M. H. M. Leung, Tak, W. Kee, D. S. English, Langmuir, 26, 5520 (2010)). Copyright (2010) American Chemical Society.

Fig. 10. Steady-state fluorescence spectra of curcumin in presence of different concentrations of DTAB in sodium phosphate buffer medium (pH = 5) at 298K [3]; Reprinted (adapted) with permission from (D. Ke, X. Wang, Q. Yang, Y. Niu, S. Chai, Z. Chen, X. An, W. Shen, Langmuir, 27, 14112 (2011)). Copyright (2011) American Chemical Society.