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The Role of Charge in the Surfactant-Assisted Stabilization of the Natural Product Curcumin

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Colloidal solutions of surfactants that form micelles or vesicles are useful for solubilizing and stabilizing hydrophobic molecules that are otherwise sparingly soluble in aqueous solutions. In this paper we investigate the use of micelles and vesicles prepared from ionic surfactants for solubilizing and stabilizing curcumin, a medicinal natural product that undergoes alkaline hydrolysis in water. We identify spectroscopic signatures to evaluate curcumin partitioning and deprotonation in surfactant mixtures containing micelles or vesicles. These spectroscopic signatures allow us to monitor the interaction of curcumin with charged surfactants over a wide range of pH values. Titration data are presented to show the pH dependence of curcumin interactions with negatively and positively charged micelles and vesicles. In solutions of cationic micelles or positively charged vesicles, strong interaction between the Cur^{-1} phenoxide ion and the positively charged surfactants results in a change in the acidity of the phenolic hydrogen and a lowering of the apparent lowest pK_a value for curcumin. In the microenvironments formed by anionic micelles or negatively charged bilayers, our data indicates that curcumin partitions as the Cur^0 species, which is stabilized by interactions with the respective surfactant aggregates, and this leads to an increase in the apparent pK_a values. Our results may explain some of the discrepancies within the literature with respect to reported pK_a values and the acidity of the enolic versus phenolic protons. Hydrolysis rates, quantum yields, and molar absorption coefficients are reported for curcumin in a variety of solutions.

I. Introduction

Colloidal solutions of surfactants that form micelles or vesicles are commonly used for solubilizing and stabilizing hydrophobic molecules that are otherwise only slightly soluble in aqueous solutions. In this paper we investigate the use of micelles and vesicles prepared from cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), cetyltrimethylammonium tosylate (CTAT) and sodium dodecylbenzenesulfonate (SDBS) for solubilizing and stabilizing curcumin, a natural product that undergoes alkaline hydrolysis in water.

Curcumin is a natural product extracted from turmeric, a principle ingredient of curry with many reported beneficial health effects.[1] Studies have shown that curcumin not only possesses anticancer properties,[2,3] but it also has anti-inflammatory,[4] antioxidant,[5] anti-Alzheimer's disease,[6] anti-cystic fibrosis,[7] and wound-healing effects.[8−10]

Here we are interested in curcumin as a prototypical model for investigating the role of surfactants as stabilizing agents with respect to alkaline hydrolysis of drug molecules. Curcumin undergoes a pH-dependent degradation in alkaline solutions, with the main degradation products being trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, vanillin, and ferulic acid.[1,11,12] Wang et al. showed that degradation can be dramatically slowed in cell growth medium by addition of 10% calf serum.[11] The reduction in degradation rates can be attributed to interactions of curcumin with serum components such as serum albumin.[13] Curcumin degradation can also be diminished by interactions with surfactant aggregates such as micelles.[14] Interestingly, despite the evidence for strong partitioning of curcumin to lipid-like environments, it is poorly adsorbed through the gut with 38−75% being excreted in the feces of rats when administered orally.[15,16] Hence, in vitro studies aimed at evaluating the interactions of curcumin with membrane interfaces could prove useful to controlling degradation in water and provide insight into in vivo interactions with the intestinal tract endothelial cell walls. In this paper we study the spectroscopic properties of curcumin as a function of surfactant mixtures including anionic, cationic, and mixed-surfactant (catanionic) environments in both micelle- and bilayer-forming solutions.

Figure 1 shows the keto−enol form of curcumin. Theoretical and experimental studies indicate that the keto−enol form is the

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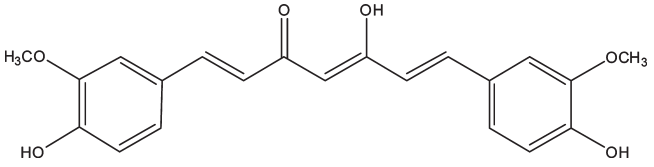

Figure 1. Keto−enol form of curcumin.

predominant isomer in solution.[17−19] Some debate exists in the literature about the pK_a values of the three titratable protons as well as which proton is the most acidic.[18,19] Literature values of the lowest pK_a range from 7.75 to 8.89.[12,18−20] In a recent report, we showed that the trianionic species, Cur^{3-}, is stabilized against alkaline hydrolysis by catanionic micelles prepared from CTAB and DTAB at pH 13. Sequestration of the Cur^{3-} from the aqueous environment occurs through electrostatic interactions with positively charged micelles,[14] which blocks hydrolysis.

In this paper we conduct spectroscopic investigations to study the interaction of curcumin with surfactant aggregates over a large range of pH values. We investigate curcumin interactions with both negatively and positively charged micelles and vesicles. We have previously reported the ability of surfactant vesicles formed from CTAT and SDBS to sequester charged organic molecules in aqueous solutions and have shown that these systems have remarkably high sequestration capacity and long-term stability, facilitated by electrostatic interactions.[21−23] Both CTAT and SDBS form micelles in water above their critical micelle concentrations (cmc's), which are 0.34 mM and 2.8 mM, respectively.[22] When CTAT and SDBS are mixed in the correct proportions, they spontaneously form vesicles that can be prepared with either a negatively or positively charged bilayer, depending on which surfactant is in excess. The critical aggregation concentration for vesicle formation is 2.6 μM due to the electrostatic attraction between the oppositely charged headgroups of CTAT and SDBS.[22] The versatility of the CTAT/SDBS system to form a range of aggregates provides a good opportunity to perform a systematic evaluation of curcumin interactions with surfactant aggregates.

II. Materials and Methods

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] was obtained from Alexis Biochemicals (purity >98.5%). Purity was confirmed by NMR and mass spectroscopy (see Supporting Information). CTAT (99%), CTAB (99%), and DTAB (99%) were obtained from Sigma, sodium dodecylsulfate (SDS) (≥99%) was purchased from Fluka, and SDBS (~95%) was obtained from TCI and used as received. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; 99%) was purchased from ACROS Organics, and methanol, ethanol, and toluene were obtained from Fisher Chemical and used as received. All aqueous solutions were prepared using deionized water from a Millipore Milli-Q NANO pure water system. Note that all of the surfactants used are either sulfonates or quaternary ammonium salts, which will remain charged over the pH range investigated.

Ultraviolet−visible (UV−vis) spectra were acquired on a Shimadzu UV2550 or Cary 5000 (Varian). Titration experiments were performed in HEPES (0.1 M) solutions (pH 4.0−12.4) by adjusting with sodium hydroxide or hydrochloric acid to obtain the desired pH. Solutions above pH 12.4 were attained by adding NaOH to pure water. The pH was measured (Acumet AB 15) before and after conducting the spectroscopic measurements. The ionic strength of the buffer was maintained at or below 0.5 M in all experiments. In this range, the ionic strength has no effect on the curcumin spectrum or hydrolysis rate. All UV−vis studies were performed with an initial curcumin concentration of 9.1 μM.

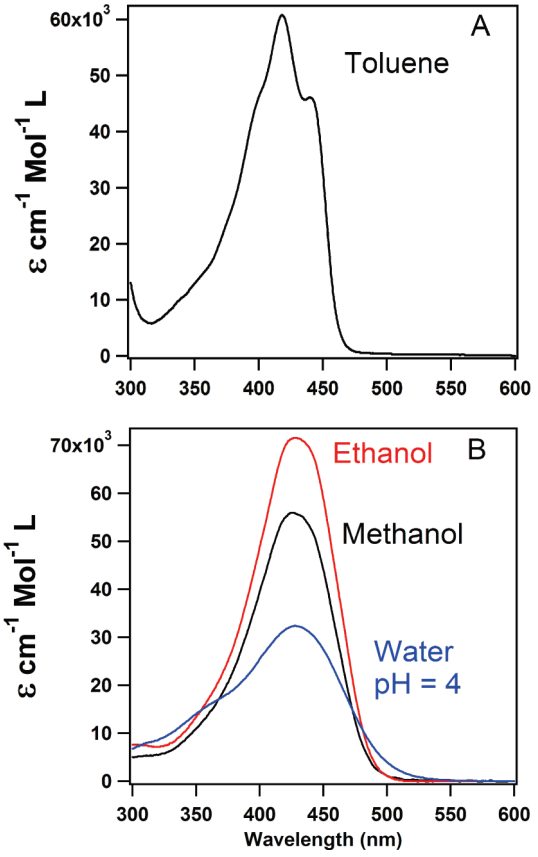

Figure 2. Absorption spectra of curcumin in different solvents.

Quantum yield measurements were performed using a Horiba Jobin Yvon Fluorolog Tau 3 spectrometer or Cary Eclipse spectrometer. Initially, curcumin in ethanol was measured relative to sulforhodamine 101 in ethanol, which has a quantum yield of 0.95.[24] The quantum yield was calculated as[25]

$$\Phi_{fl} = \frac{I^{sample}}{I^{ref}} \frac{OD^{ref}}{OD^{sample}} \left(\frac{n^{sample}}{n^{ref}} \right)^2 \Phi_{fl}^{reference} \quad (1)$$

where I is the integrated intensity of the corrected emission spectrum, OD is the optical density in absorbance units at the excitation wavelength, and n is the index of refraction.

The quantum yield for curcumin in ethanol was determined with sulforhodamine as the reference, and all other quantum yields were determined with curcumin in ethanol as the reference.

III. Results and Discussion

The curcumin absorption spectrum has a strong solvent dependence. In nonpolar, aprotic solvents, the maximal extinction coefficient ranges from 50 000 to 60 000 $cm^{-1}L\ mol^{-1}$, and the spectrum displays vibronic structure with λ_{max} near 420 nm, as

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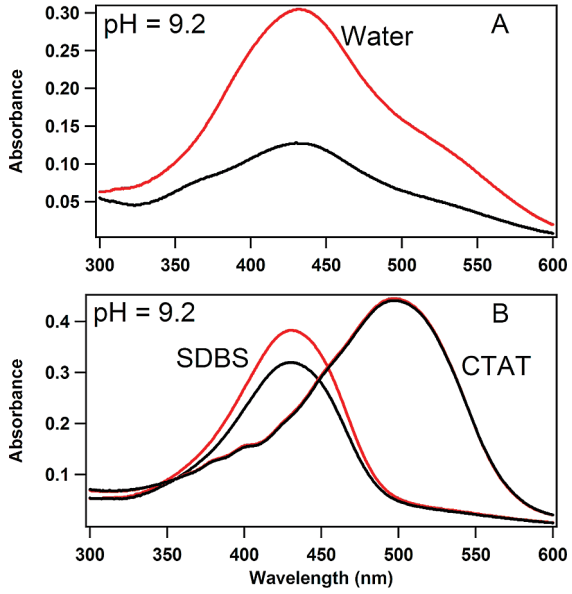

Figure 3. Spectroscopic measurements of alkaline hydrolysis at pH 9.2. Spectra were taken of the fresh sample (red line), and 1 h later (black line) (A) in water and (B) in micelles formed from SDBS (4 mM) and CTAT (4 mM). Curcumin concentration was 9.1 μM in all experiments.

shown in Figure 2A. These features correspond to the fully conjugated form of the protonated enol.[18,19]

In protic, polar solvents (Figure 2B), λ_{max} shifts to ~430 nm, the vibronic features are no longer resolved, and the molar absorptivity decreases as solvent polarity increases. The spectra in ethanol, methanol, and water (pH 4) shown in Figure 2B, are representative of the hydrogen-bonded enol. In water, over the range of pH 6−12, curcumin undergoes deprotonation at the enol and at both phenol sites, changing from a neutral molecule to a trianionic species. Using UV−vis, we measure the lowest apparent pK_a value of curcumin in water to be 8.3, (discussed below). This value corresponds well with previous measurements by Bernade-Pineda et al. who have shown the deprotonation of the enol group occurs with a pK_a of 8.4 and they assign pK_a values of 9.9 and 10.5 for the phenol groups.[26] Using these values, we have estimated the average charge of curcumin as a function of pH and found that curcumin has a net charge of −1 at pH 9.1−9.2 (see Supporting Information for calculations and speciation diagram). At pH 9.2, approximately 75% of curcumin is present as Cur^{-1}.

Figure 3 shows the absorption spectra for curcumin in neat water and in micelle solutions at pH 9.2. In water at pH 9.2, deprotonation to form Cur^{-1} leads to an increase in absorbance at $\lambda > 490$ nm and a corresponding decrease at $\lambda < 450$ nm. The spectra in Figure 3 were taken from the freshly prepared curcumin solutions (red lines) and 1 h after addition of curcumin (black lines). Figure 3A shows a large decrease in absorbance over the course of 1 h, as curcumin undergoes alkaline hydrolysis.

Figure 3B shows curcumin in CTAT and SDBS micelles at pH 9.2. In 4 mM SDBS, curcumin degrades with an initial rate of 0.43 nM s^{-1}, which is substantially lower than the rate in water, 3.3 nM s^{-1}, as shown in Table 1. The reduced degradation rate in SDBS and the high quantum yield (Table 1) relative to water shows that there is significant interaction between curcumin and SDBS micelles. At pH 9.2 the absorption spectrum in SDBS micelles differs from the spectrum acquired in water but is nearly identical to that in methanol or ethanol with only a slight mismatch occurring at wavelengths above 490 nm. The spectrum indicates that the curcumin population in SDBS micelles is dominated by the hydrogen bonded enol, Cur^0, observed in alcohols. This population is in equilibrium with the deprotonated curcumin species that give rise to the increased absorbance above 490 nm relative to alcohols. The quantum yield observed in SDBS micelles is much higher than any other micelle mixtures in Table 1. In fact it is nearly as large as the quantum yield observed in ethanol, providing further evidence that, at pH 9.2, curcumin partitions to the anionic micelle as the protonated enol, Cur^0. This species dominates both the absorption and emission characteristics of the sample. The fraction of curcumin free in solution as Cur^{-1} will undergo hydrolysis at the normal rate. As Cur^{-1} degrades, the partitioning equilibrium adjusts, and the overall degradation is only weakly diminished in spite of the obvious interactions with the micelles.

The observations for curcumin at pH 9.2 in the presence of cationic micelles is distinctly different from that of anionic micelles. As shown in Figure 3B and Table 1, CTAT micelles cause a strong red shift in the absorption spectrum and halt the degradation process. Identical observations are made for curcumin in CTAB and DTAB micelles. The observed stabilization with respect to hydrolysis indicates that all of the curcumin has partitioned to the cationic micelle phase where the reaction pathway is blocked. The strong red shift suggests a distinct molecular species or solvent environment different from any of the others represented in Figures 2 and 3. Insight into the origin of this large red shift in CTAT, CTAB, and DTAB micelles can be found from the experimental and theoretical studies published previously.[18,19]

Computational modeling by Shen and Ji shows that only deprotonation of the phenol group can result in a large red-shift.[18] Experimentally, this finding is supported by the work of Zsila et al. in which curcumin and a curcumin analogue 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one], or half-curcumin, were investigated using UV−vis spectroscopy. Each of these molecules show a large red shifts of ~5000 cm^{-1} upon addition of potassium hydroxide in ethanol.[19] Drawing from the data acquired with curcumin, half-curcumin, and other curcumin analogues, Zsila et al. showed that the wavelength position of the absorption spectrum is only weakly affected by deprotonation of the enol but that deprotonation of a *para* positioned phenolic OH group results in a large red shift. In dioxane/water mixtures, curcumin analogues with one or two *para* positioned OH groups undergo a 3000−5000 cm^{-1} red shift when one or both of the hydroxyl groups are deprotonated by the addition of base.

In our experiments using CTAT, CTAB, or DTAB micelles, a red shift of 3400−3600 cm^{-1} is observed in going from pH < 7 to pH 9.2. The fact that this large shift is observed in CTA^+ and DTA^+ micelles at pH 9.2 but not in anionic micelles or water indicates there is a strong interaction between curcumin and CTA^+/DTA^+ that stabilizes the phenoxide ion. Figure 4 shows curcumin in aqueous buffer, where a significant red-shift is observed only at pH values greater than 10. In this pH range, the Cur^{-2} and Cur^{-3} species begin to dominate and result in an increase in the large red-shifted band. This observation strongly suggests that the phenolic hydrogens are removed after the enolate formation. Hence, we argue that, in water, the enolic proton is most acidic. Our major observation in cationic micelles is a reversal in the order of deprotonation between cationic micelles and water: *in the presence of CTA^+ or DTA^+ micelles, the phenolic hydrogen is most acidic.*

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Table 1. Curcumin Hydrolysis Rates[a]

surfactant (molarity)		initial rate of curcumin hydrolysis (nM s^{-1})	fluorescence quantum yield, Φ_{fl}	decadic molar absorption coefficient (L $mol^{-1}cm^{-1}$)
toluene		stable[b]	2×10^{-1}	5.0×10^4
ethanol		stable	8×10^{-2}	
methanol		stable	3×10^{-2}	
water	pH = 4.0 (Cur^0)		1×10^{-3}	3.0×10^4
	pH = 4.7		1×10^{-3}	
	pH = 7.3	10.5 ± 0.3	1×10^{-3}	
	pH = 9.2	3.33 ± 0.3	8×10^{-4}	
	pH = 12.3	0.23 ± 0.04		
	pH = 13.7 (Cur^{-3})	0.052 ± 0.002	2×10^{-4}	4.9×10^4
SDBS (4 mM)	pH = 9.2	0.43 ± 0.05	3×10^{-2}	4.3×10^4
	pH = 12.4	0.059 ± 0.007	3×10^{-4}	
SDS (16 mM)	pH = 9.2	0.11 ± 0.03	7×10^{-3}	
	pH = 13.7	0.012 ± 0.002	7×10^{-5}	
CTAT (4 mM)	pH = 9.2	stable	9×10^{-3}	4.9×10^4
	pH = 12.3	0.16 ± 0.03		
	pH = 13.7	0.022 ± 0.001	2×10^{-3}	
CTAB (4 mM)	pH = 13.7	0.084 ± 0.003	2×10^{-3}	
DTAB (31 mM)	pH = 9.2	stable	6×10^{-4}	
	pH = 13.7	0.026	2×10^{-3}	
SDBS:CTAT (3:1) pH = 9.2 (0.92 mM)		0.66 ± 0.04	1×10^{-1}	4.1×10^4
CTAT:SDBS (1.8:1) pH = 9.2 (0.37 mM)		0.44 ± 0.03	9×10^{-3}	4.8×10^4

[a] Details of hydrolysis rate calculations can be found in the Supporting Information. [b] The solution was deemed stable if no measurable degradation is observed over the course of 1 h while monitoring with UV−vis absorption spectroscopy.

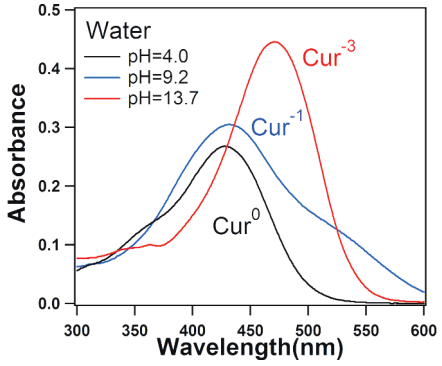

Figure 4. Curcumin absorption spectrum in water at pH 4 (black line), pH 9.2 (blue line), and pH 13.7 (red line).

In cationic micelles at pH 9.2, our spectroscopic evidence points toward the monoanionic phenoxide that forms an ion pair with the CTA^+ cation. This interpretation is consistent with diminished degradation and the large red shift. The fluorescence quantum yield of curcumin in CTAT micelles at pH 9.2 is 3 times less than that in SDBS micelles and 10 times grater than in water. The change in fluorescence yields when micelles are present at pH 9.2 clearly shows that curcumin significantly partitions to both types of micelles under these conditions. The high quantum yield for curcumin in SDBS micelles is comparable to the yield in ethanol (see Table 1) and can be assigned to the Cur^0 species residing in DBS^- micelles. The lower fluorescence quantum yield of curcumin in CTA^+ is consistent with the phenoxide species since a decrease of quantum yield is observed in water, cationic micelles, and anionic micelles at pH > 10 (Table 1).

To summarize, our observations of curcumin in micelle solutions at pH 9.2 suggest two different modes of interaction, which depend on the micelle charge. Our findings indicate that the phenoxide monoanion interacts strongly with positively charged CTA^+ or DTA^+ micelles through electrostatic and hydrophobic interactions. This results in nearly total partitioning to the micelle phase, essentially blocking the degradation pathway. In SDBS micelles, curcumin partitions to the micelle in its neutral form, where it is highly fluorescent but remains in equilibrium with the enolate in solution; as a result, the degradation rate is only slightly reduced.

To expand on these observations, we conducted spectrometric titrations of curcumin in both cationic and anionic micelles, as shown in Figure 5. During titration, the spectrum of curcumin undergoes fairly complex changes with no observed isosbestic points. To capture these changes in a single quantity, we calculated a spectral mean, i.e., first moment, and plotted this value against pH. The absorption spectral mean was calculated as

$$\text{Absorption spectral mean} = \overline{\lambda} = \frac{\sum_{\lambda=300\text{nm}}^{\lambda=600\text{nm}} \text{OD}(\lambda)\cdot\lambda}{\sum_{\lambda=300\text{nm}}^{\lambda=600\text{nm}} \text{OD}(\lambda)} \quad (2)$$

where OD is the optical density at a given wavelength, λ. For curcumin in water a clear sigmoidal change is observed in the spectral mean (+ symbol in Figure 5A and 5B). The half point of the sigmoid occurs at pH = 8.3, which is in close agreement with the midpoint of the literature values for curcumin's lowest pK_a in water, which range from 7.75 to 8.89. Hence, the absorption spectral mean is a useful observable for recording the lowest pK_a of curcumin. Even at acidic pH values, the absorption spectra and fluorescence quantum yields of Cur^0 in micelle solutions differ from water. This indicates that Cur^0 partitions to ionic micelles, regardless of their charge. As the pH increases, each of the curves in Figure 5 undergoes a sigmoidal transition. Figure 5A shows that, for curcumin in 4 mM CTAT (red squares), CTAB (blue squares), and DTAB (green squares) at low pH values, the spectral mean is slightly red-shifted from aqueous curcumin as a result of interactions of Cur^0 with the micelles. When the pH is raised, the sigmoidal half point occurs at pH 7.6 and 7.8 for DTAB and CTAB, respectively. These values are much lower than in water. This observation is consistent with strong interactions between Cur^{-1} and CTA^+ or DTA^+ that effectively stabilize the curcumin anion and shift the acid base equilibrium to a lower pK_a value. In CTAT, the pK_a is nearly unchanged, and this is due

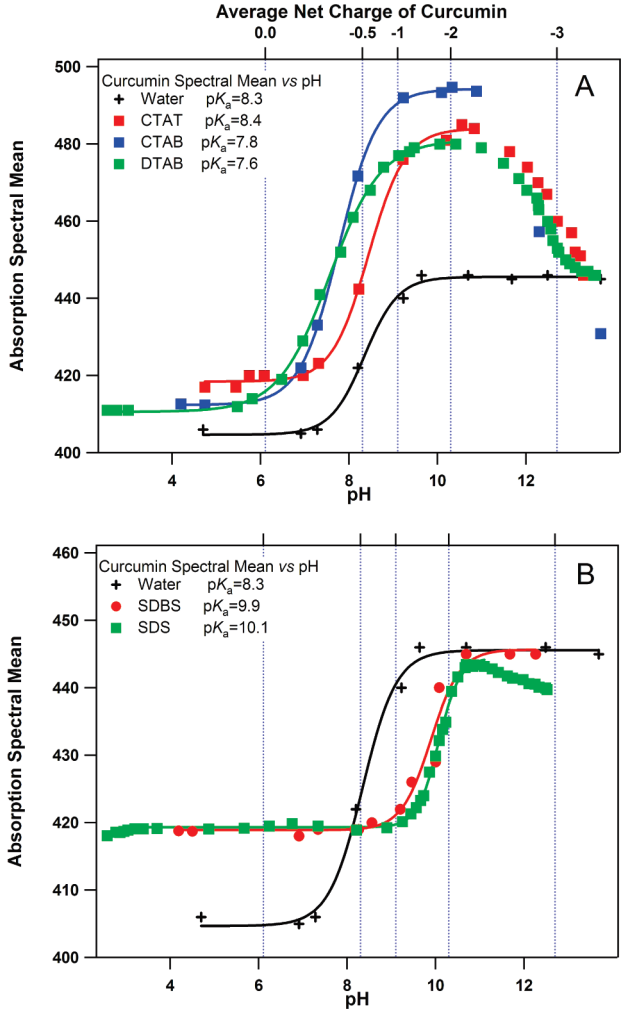

Figure 5. Curcumin titration experiments in micelles compared to pure buffer. (A) CTAT, CTAB and DTAB micelles. (B) SDBS and SDS micelles.

to the presence of the tosylate ion, which competes with Cur^{-1} for direct ion pairing with CTA^+. The behavior of curcumin in all three cationic micelles is very similar, as shown in Figure 5A and Table 1. Due to this similarity, it is unlikely that cationic micelle shape plays a large role in stabilizing curcumin, since DTAB and CTAB will form spherical micelles and CTAT will form elongated micelles.[27−29]

The titration curves obtained in CTA^+ and DTA^+ micelles become nonsigmoidal above pH 11.2. The top axis in Figure 5 shows the average charge of curcumin in water as a function of pH (see Supporting Information for calculations). The decrease in spectral mean might be explained by dissociation of the di- and trianionic curcumin from the cationic micelles due to increased water solubility, but this conclusion is not supported by the observed hydrolysis rates ,which remain relatively small in cationic micelles throughout the entire pH range (Table 1). Instead, the convergence of the spectral mean in cationic micelles with the aqueous values at high pH can be explained by deprotonation of the enol and both phenols to create the trianion, which has a similar absorption spectrum in all three environments.

Figure 5B shows a comparison between curcumin titrations in water and in SDBS or SDS micelles. At low pH values the spectral mean in anionic micelles matches well with the data from cationic micelles, indicating equivalent partitioning of Cur^0 to both anionic and cationic micelles. In contrast with cationic micelles, anionic micelles give a much higher pK_a value than that observed in water. In both SDBS and SDS micelles, the observed pK_a values are >1.5 pH units above that of curcumin in water. This observation is consistent with the stabilization of the neutral curcumin species by the anionic micelles. This effect is due to charge and not tail length or micelle size since SDS and SDBS give nearly identical titration data. In SDBS micelles, the spectral mean values match those from water at pH values above 10.7, and the absorption spectra themselves overlap well. Furthermore, the hydrolysis rates and fluorescence quantum yields for curcumin in water and SDBS micelles are nearly identical at higher pH values. Over the range of pH 9.2−13, the average curcumin charge goes from −1 to −3. Once the pH is high enough to overcome the stabilizing effects of SDBS micelles on Cur^0, dissociation of the various charged curcumin species occurs, and this is reflected in the hydrolysis rate, which closely matches the rate in water at pH 13.7. The spectral mean of curcumin in SDS solutions at high pH does not match that of water and SDBS micelles, suggesting that there may be some residual interactions between the SDS micelles and Cur^{-2}/Cur^{-3}. This may also be due to the higher concentration used for SDS (>8 mM).

In summary, our titration experiments show the stabilizing effects of CTA^+ micelles on Cur^{-1} versus the stabilizing effects of DBS^- micelles on Cur^0. These stabilizing effects result in the observed pK_a values given in Figure 5. The ability to selectively stabilize specific ionic species might be used to provide access to specific chemical pathways to carry out functionalization of curcumin or similar molecules.

Curcumin Interactions with Vesicles. CTAT and SDBS were chosen for these studies since, in the right compositions, they form well-characterized unilamellar vesicles and provide a convenient means to compare curcumin in micelles with curcumin in a charged *bilayer* without changing components. In terms of potential applications, an advantage of using CTAT/SDBS mixtures is that the critical aggregation concentrations are 2−3 μM, well below the cmc's of the individual components, and therefore less surfactant is needed to sequester curcumin. These vesicles are referred to as catanionic vesicles since their formation is due to the formation of cation/anion pairs. These ion pairs have packing geometries similar to a diacyl lipid and favor the formation of a lamellar aggregate.[22,30,31] Vesicles only form in mixtures where one of the components is in excess, and this imparts a net charge to the vesicle bilayer. We have studied molecular sequestration by vesicles formed from two different compositions of SDBS and CTAT consisting of (1) a 3-fold molar excess of SDBS to CTAT and (2) a 1.8-fold molar excess of CTAT to SDBS.[21−23] Each of these compositions forms vesicles in which the excess surfactant is solubilized in the vesicle bilayer and the vesicles are charged, with zeta potential of approximately ±50 mV. The bilayer is composed of a mixture of neutral ion-paired surfactants, similar to zwitterionic lipid molecules, and unpaired molecules of the excess surfactant. The unpaired molecules are responsible for the bilayer charge and the excellent colloidal stability exhibited by catanionic vesicles.

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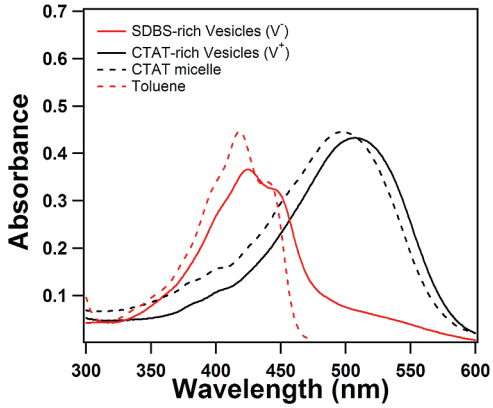

Figure 6. Curcumin interactions with surfactant vesicles. Absorption spectra of curcumin in SDBS-rich and CTAT-rich catanionic surfactant vesicles and comparison with CTAT micelles and toluene.

The absorption spectra for curcumin in catanionic vesicles at pH 9.2 are shown in Figure 6. In CTAT-rich vesicles at a total surfactant concentration of 0.37 mM, the spectrum is similar to that in CTA^+ micelles but slightly more red-shifted, and the fluorescence quantum yield is identical in both samples. In CTAT-rich vesicles, the CTA^+ ions are distributed in the bilayer as opposed to being aggregated together as they are in a CTA^+ micelle. In CTAT-rich vesicles, each Cur^{-1} is interacting with a single CTA^+ ion isolated in the vesicle bilayer, and this may explain the slight differences between the curcumin spectrum in CTAT micelles and CTAT-rich vesicles shown in Figure 6. In vesicle samples, the excess CTA^+ ions can also exchange between pairing with Cur^{-1}, tosylate, and DBS^{-1}, hence the Cur^{-1}/CTA^+ ion pair may be more facile in the vesicle, and this can explain the measurable rate of hydrolysis observed in CTAT-rich vesicles relative to CTA^+ micelles in which no hydrolysis is observed.

In SDBS-rich vesicles at pH 9.2 with a total surfactant concentration of 0.92 mM, curcumin gives rise to a spectrum that shows the vibronic structure observed in nonpolar, aprotic solvents. In fact, the spectrum in SDBS-rich vesicles is remarkably similar to the spectrum acquired in toluene. This is due to the nature of the vesicle bilayer where CTA^+ ions have paired with DBS^- ions to form a bilayer composed of neutral ion-pair amphiphiles with the excess DBS^- distributed throughout the bilayer. Note also that Cur^{-1} is water-soluble, and without the electrostatic interactions with CTA^+ it will not partition to the negatively charged SDBS-rich bilayer. At pH 9.2, when curcumin partitions to the SDBS-rich bilayer, it partitions as Cur^0. Hence, Cur^0 resides in a bilayer consisting of alkyl-substituted benzene sulfonate groups, which emulate the toluene environment. As with SDBS micelles, Cur^0 in the bilayer is in equilibrium with aqueous Cur^{-1}, and the presence of solvated Cur^{-1} gives rise to the red-shifted "tail" in the absorption spectrum of Figure 6 and also to the rapid hydrolysis rate. Importantly, curcumin in SDBS-rich vesicles has the highest fluorescence quantum yield that we measure in any surfactant solution, and it is nearly as large as that of curcumin in toluene. This observation coupled with the shape of the absorbance spectrum provides strong evidence that the species present in SDBS-rich vesicles is the Cur^0 molecules buried in the nonpolar, toluene-like bilayer interior.

Titration experiments in the two vesicle mixtures were also carried out, and these results are shown in Figure 7. Curcumin in CTAT-rich vesicles behaves similarly to CTAT micelles but

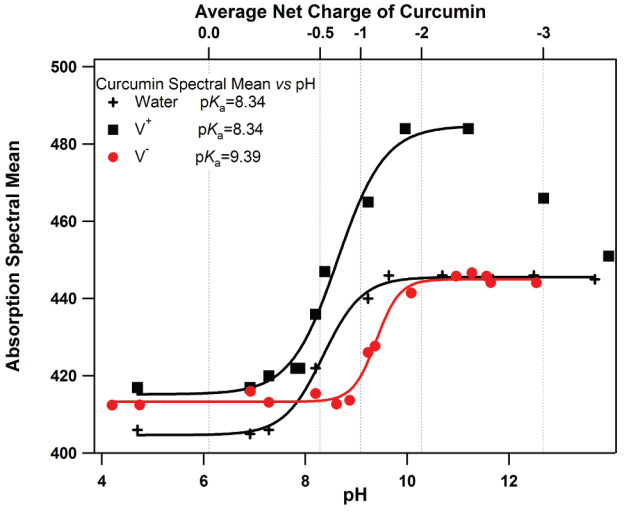

Figure 7. Curcumin titration experiments in CTAT-rich and SDBS-rich vesicles. Total surfactant concentration was 0.4 mM for V^+ and 0.9 mM for V^-.

with spectral mean values that are intermediate between those observed for micelles and water at low pH values. From simple stoichiometry, 55% of the CTA^+ in the vesicle bilayer can pair with DBS^-, but a significant fraction of the remaining CTA^+ has been shown to bind strongly with anions in solution, including small molecules and DNA.[21−23] However, Cur^{-1} must compete with the tosylate and DBS^- ions, and therefore the stabilizing effects are not strong enough to measurably shift the observed pK_a. In SDBS-rich vesicles, the pK_a is shifted higher as was observed for SDS and SDBS micelles and is consistent with stabilization of the Cur^0 species by sequestration in the bilayer. Note that the pK_a in SDBS-rich micelles is intermediate between water and anionic micelles, indicating that the stabilizing effect is weaker for vesicles. This is further reflected in the hydrolysis rates, which are higher for both vesicle mixtures when compared to rates obtained in analogous micelle solutions.

IV. Conclusions

We have shown a number of interesting findings with respect to sequestration of curcumin by surfactants. If the desired result is to stabilize curcumin against alkaline hydrolysis, cationic surfactants are far superior to anionic surfactants, particularly in the pH range where Cur^{-1} is the dominant species. At this pH range, the excellent stabilization arises from strong interactions between the phenoxide ion and the cationic surfactant. Hence, when interacting with cationic surfactants at pH 9.2 the degradation process is completely inhibited and curcumin is then available for synthetic transformations such as methylation of phenoxide ion that would otherwise be unachievable in water. This opens up the possibility of using surfactants as a "green" catalyst for performing reactions in water that would otherwise require organic solvents.

We have also demonstrated the utility of using the absorbance spectral mean to evaluate the deprotonation of curcumin in a variety of surfactant solutions. Our results show that the spectral mean can be used to monitor changes in the apparent pK_a of curcumin as a function of the surfactant environment. The strong interaction of Cur^{-1} with CTA^+ and DTA^+ ions results in a lowering of the apparent pK_a values when micelles with bromide counterions are used. In SDS and SDBS micelles or SDBS-rich vesicles, the Cur^0 species is stabilized by interactions with the

respective surfactant aggregates, and this leads to an increase in the apparent pK_a values.

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Supporting Information Available: Mass spectrometry and NMR characterization of curcumin along with acid−base equilibrium calculations and details of hydrolysis rate determinations. This material is available free of charge via the Internet at http://pubs.acs.org.