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Effect of Micellar and Reverse Micellar Interface on Solute Location: 2,6-Pyridinedicarboxylate in CTAB Micelles and CTAB and AOT Reverse Micelles

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The interface−solute interactions, including solute location, surfactant charge, and geometry of solute interactions were studied in CTAB micelles and reverse micelles and were found to be similar as measured using ^{1}H NMR spectroscopy and a pH-sensitive probe. ^{1}H NMR spectra were recorded in the presence and absence of 2,6-pyridinedicarboxylate probe at CTAB concentrations above and below the critical micelle concentration showing interaction between dipic-probe and the micellar self-assembled structure. Downfield chemical shifts are observed for the CTAB surfactant signals upon aggregation and micelle formation. The effect of micelle formation on CTAB chemical shifts was quantitated, and simple ion pairing was ruled out. No significant change in CTAB surfactant signals are observed in the presence of monoanionic probe, whereas significant shifts are observed in the presence of the dianionic probe. The ^{1}H NMR spectra of the dipic-probe are diagnostic of the protonation state and isomeric form of the dipic-probe. The ^{1}H NMR chemical shifts in micelles are sensitive to the location of the dipic-probe, and the downfield chemical shift suggests location of part of the molecule in the Stern layer near the charged interface. Other parts of the probe show an upfield chemical shifts consistent with a deeper penetration of the dipic-probe into the surfactant layer. Probe location was confirmed using the 2D ROESY. Spectra recorded of the dipic-probe at various pH values demonstrate that both CTAB micellar and CTAB/pentanol/cyclohexane reverse micellar interfaces are different than those reported in aqueous solution and in AOT/isooctane reverse micelles (Crans et al. *J. Org. Chem.* **2008**, *73*, 9633−9640) and suggest interface penetration by dipic^{2-}. Together these observations and comparisons provide guidelines for future interpretation of chemical shift changes in both micelles and reverse micelles and point to headgroup charge as being a key factor determining the direction of chemical shift change and the depth of solute penetration.

Introduction

Microemulsions are attractive alternatives to conventional drug formulations as demonstrated by the success of the cyclosporine microemulsion formulation Neoral.[1] Noncovalent assemblies based on water-in-oil, oil-in-water, and other self-assembled lipid systems are used as effective drug delivery vehicles.[2−5] For example, nonionic micellar and microemulsion systems based on surfactant Brij 96 and soybean oil were used to incorporate testosterone and progesterone.[6] The drug loading in the microemulsion formulation was found to be higher than a micellar system, since the drug loading was related to the drug lipophilicity.[6] We have demonstrated that simple metal complexes[7] and carboxylic acids[8] are able to penetrate surfactant interfaces even though they are charged and generally would be expected to reside in the water pool.[9] These studies are in agreement with the emerging literature using a range of methods, also demonstrating that some of these agents readily penetrate deep into the interior of surfactant interfaces both in model systems[10,11] and in lipid bilayers.[12−14] Together these studies suggest that information allowing a detailed comparison of the environment of such simple probes would be valuable and provide guidelines into the current approaches used for development of successful drug formulations.

Important biological processes take place at interfaces or in confined environments, and micellar and reverse micellar interfaces (Figure 1) provide simplified models retaining the essential Coulombic and hydrophobic interactions important for interface interaction with probe molecules. Multiple studies have been reported characterizing the interactions of probes with interfaces and waterpools[15] with for example a detailed comparison of charge and confinement effects on ions in reverse micelles.[16,17] Few studies have directly compared micelles and reverse micelles, so limited information is available on their abilities to solubilize various types of solutes.[18] The formation of 5-hexadecyl-7-methylindazole from 2,6-dimethyl-4-hexadecylbenzenediazonium tetrafluoroborate in the cationic CTAB micellar interface have been interpreted in terms

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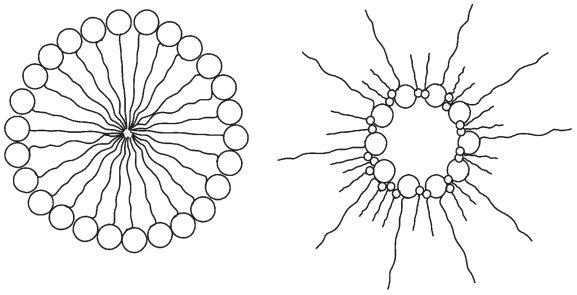

Figure 1. Schematic representation of a CTAB micelle (left) and a reverse micelle (right).

of significantly higher basicity of micellar interface compared to the CTAB reverse micellar microemulsion interface in isooctane with 1-hexanol as a cosurfactant.[19] The higher basicity of the CTAB micellar interface was attributed to the lower counterion concentration near the interface.[19] Nitroso group transfer from *N*-methyl-*N*-nitroso-*p*-toluensulfonamide to various secondary amines was compared in DTAB micelles, AOT reverse micelles, and DODAC vesicles.[18] When complex bimolecular reactions were analyzed assuming reactant distribution in the pseudophase, the authors were able to demonstrate that the reactive position in the interface changes with the amine hydrophobicity.[18] Interface charge and confinement increased the electron transfer rate between a Co(3+) complex and aqueous Fe(2+) ion in both CTAB micelles and AOT reverse micelles compared to aqueous solution.[20] Since the charges on the CTAB micelles and AOT reverse micelles are different, these studies imply that confinement is more important than surfactant charge for this reaction. Although solute and water may sometimes respond differently, in a recent review[15] Fayer and Levinger concluded that for water in reverse micelles the confinement effect is more important than the interface charge. Finally, the rotational correlation of nitroxide labeled 5-doxyl stearic acid in CTAB micelles and microemulsions was determined using EPR spectroscopy and the nitroxide tumbling correlation time was consistent with the probe residing in the interface.[21] The probe rotation in the reverse micelle was faster than in the micelle, consistent with the known properties of different sizes of reverse micelles, the aggregation at higher concentration and potential probe distribution in the pseudophase and organic solvents.[22]

Dipicolinic acid, 2,6-pyridinedicarboxylic acid, is a simple flat aromatic acid that effectively chelates metal ions in aqueous solution and has been characterized in detail.[8,23,24] The ligand itself presents an interest because its calcium complex[25] is known to exist naturally in the spores of *Bacillus* and *Clostridium* genera including Anthrax, where it makes up 20−30% of the coat weight.[26] Since this natural metabolite has an important function, characterization of its fundamental properties such as how it interacts with lipids and interfaces may be important to the efflux of dipic from its confinement in the spores. It has been shown that efflux of cations, Ca-dipicolinate, and free amino acids is critical to the signal that allows rapid influx of water into the core.[26] Several studies into the mechanisms of dipic release[27] and uptake[28] have recently been undertaken, but further detail of how it transverses the spore and spore coat is not yet known.

Many factors affect the ^{1}H chemical shifts in aqueous solution, including the dipic protonation state (Figure 2), possible stacking, isomer preference, as well as the location environment.[8,23] Upon protonation of dipic^{2-} to Hdipic$^-$ normal downfield shift was observed, consistent with deshielding by reducing electron density from the H-atoms in the molecule. Precedent exists for pyridine and related molecules that nitrogen protonation results in downfield shifts as monitored by ^{1}H NMR spectroscopy and upfield shifts when monitored by ^{15}N NMR spectroscopy.[30] In contrast, protonating Hdipic$^-$ to H_2dipic is accompanied by the upfield chemical shift. The reported upfield shift upon Hdipic$^-$ protonation in aqueous solution[8] shifts the "wrong way" and is suggestive of other factors impacting the chemical shifts in these mixtures. Dimerization,[8] changes in isomer population, or in pK_a values[31] could readily account for such usual observations and must be addressed by appropriate control experiments. A study of this biologically important and versatile probe near the charged interface in micellar and reverse micellar environment provides valuable insights into this environmental effect on its protonation, charge distribution, and isomer partitioning.

The ^{1}H NMR chemical shifts of substituted benzoates have been used to characterize the molecule location at the micellar interface.[33−35] Probe penetration into the micellar carbon chain (palisade) region results in additional shielding by the nonpolar environment and causes an upfield shift as compared to aqueous solution. Probe location at the highly charged (Stern) layer of the polar headgroups results in deshielding and downfield chemical shift. In reverse micelles, the observations have been less consistent. Downfield ^{1}H NMR chemical shifts have been observed for complexes penetrating the surfactant interface,[7,8] and have been used to infer location of other complexes.[36] A small upfield shift, however, was observed for H_2dipic in AOT/isooctane reverse micelles at pH 1.6 for the H_b proton.[8] Since pH probes such as dipic often change protonation states, studies determining probe location based on ^{1}H NMR spectroscopy must consider the effect of changes in protonation state as well as probe location. Other NMR active nuclei such as ^{51}V have been used to study reverse micelles and differences in probes depending on the headgroup charge in the reverse micelles exist.[7,37] The observations in micelles are consistent and give an upfield shift in the ^{1}H NMR shift upon probe penetration, however, in reverse micelles the results suggest a downfield chemical shift upon probe penetration. A detailed comparison between dipic probe behavior in micelles and reverse micelles is warranted.

This work was specifically designed to provide a comparison between solute interaction with the surfactant interface in both micelles and reverse micelles and consider multiple topics such as solute location, surfactant charge, and geometry of solute interactions. We used the pH-sensitive dipic probe and the CTAB surfactant. We conducted 1D and 2D ^{1}H NMR studies to obtain

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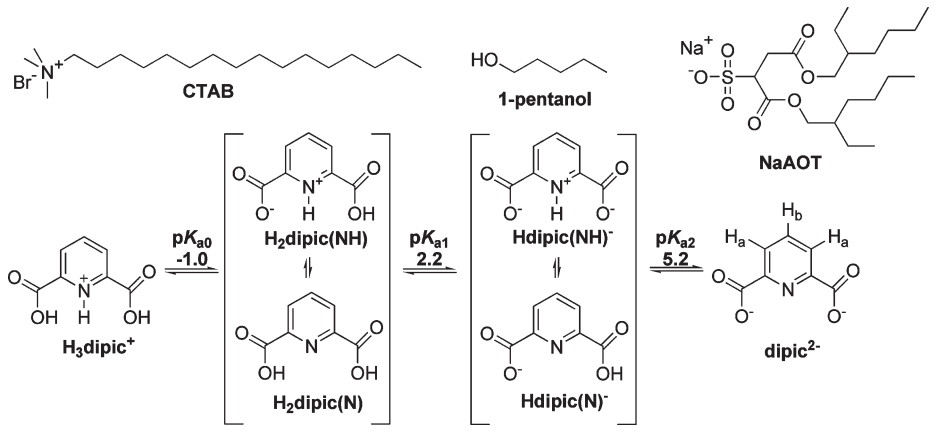

Figure 2. Structures of the cationic surfactant CTAB, cosurfactant 1-pentanol, anionic surfactant AOT, and probe molecule 2,6-pyridinedicarboxylic acid (H_2dipic). Protonation equilibria of H_2dipic in aqueous and micellar solutions are shown. Nitrogen-protonated neutral molecule H_2dipic(NH) is only of hypothetical importance, since there is no spectroscopic evidence for its formation. Previously reported[29] pK_a values in aqueous solutions are shown.

the spectroscopic changes for both CTAB micelles and CTAB reverse micelles. A 2D ROESY spectrum confirmed the interpretation that dipic^{2-} penetrates the interface in micelles. Since the solute is pH-sensitive, pH studies were undertaken to identify pH effects. The observed chemical shift changes upon micelle formation in the presence and absence of probe served to report on the extent of solute penetration and how penetration varied with solute charge. In addition, these chemical shift changes were compared to those in reverse micelles. Other techniques were used when appropriate and UV−vis data was found to confirm our conclusions obtained from NMR results. These experiments provide benchmark data that can be used for future studies in either micelles or reverse micelles using ^{1}H NMR spectroscopy.

Experimental Section

Materials. CTAB (99%, Aldrich) was purified by recrystallization from anhydrous ethanol.[38] Cyclohexane (HPLC grade, Fisher), 1-pentanol (≥99.5%, Aldrich), SDS (Ultrapure, US Biochemical Corp.), and 2,6-pyridinedicarboxylic acid (99%, Aldrich) were used as purchased. Deionized water (≥17 MΩ) was used throughout.

Methods. The ^{1}H NMR spectra of micellar solutions were acquired on a Varian Inova 300 operating at 299.953 MHz frequency at 35 °C using coaxial internal capillary filled with d_6-benzene for deuterium lock and TMS for the reference (δ = 0.000 ppm). Chemical shifts were reproducible within 0.005 ppm when recorded at 35 °C, above the Krafft temperature.[39] Initial studies below the Krafft temperature show much greater variation as expected if the micellar system is aggregating.

The ^{1}H NMR spectra of reverse micellar solutions were recorded on a Varian Inova spectrometer operating at 400.109 MHz at ambient temperature (23.7 ± 0.2 °C) in the unlocked mode. Spectra were referenced either against internal TMS or versus the cyclohexane resonance (δ = 1.443 ppm). Samples for 2D NMR spectroscopy were prepared using d_{12}-cyclohexane.

UV−vis spectra were acquired on a Perkin-Elmer Lambda 25 UV/vis spectrometer at 35 °C using aqueous surfactant solution as a blank. Solution pH was measured with Orion 720A+ pH-meter equipped with Metrohm combination pH electrode calibrated in three buffer solutions (pH's 4.0, 7.0, and 10.0).

Routine Characterization. The micellar systems were prepared according to known procedures,[40,41] and their micellar properties confirmed using DLS and optical spectroscopy. The reverse micellar preparations were tested using conductivity and DLS and were found to be in accord with literature.[40,41]

Micellar Solutions. Micellar solutions were prepared by dissolving purified CTAB and H_2dipic in deionized H_2O and adjusting solution pH at ambient temperature. Samples were stored at 35 °C overnight, and ^{1}H NMR spectra were acquired. Solution pH's were rechecked after the NMR spectra acquisition, and the difference in all cases was smaller than 0.05 pH units.

Reverse Micellar Solutions. Each sample was prepared separately by combining purified solid CTAB, 1-pentanol, cyclohexane, and the aqueous 23 and 50 mM dipic stock solution. CTAB and 1-pentanol concentrations in cyclohexane before the addition of aqueous phase were 0.15 and 0.75 M, respectively, and the molar ratio [H_2O]/[CTAB] (w_0) was equal to 6, unless specified otherwise. All samples were transparent, single phase solutions throughout the experiments.

Results and Discussion

Dipic in CTAB Micelles: pH Variation. ^{1}H NMR spectra of dipic acquired in CTAB micellar solutions in the pH range from 1.5 to 11.8 (Figure 3) show a completely different pH profile than observed in aqueous solution.[8] With increasing solution pH, both H_a and H_b proton signals shift upfield, except within the narrow pH range from 1.5 to 2.5, where H_b shifts downfield. The upfield chemical shifts are typical for deprotonation reactions.[42] In aqueous solution, Hdipic$^-$ deprotonation to dipic^{2-} is accompanied by the usual upfield shift, whereas H_2dipic deprotonation to the Hdipic$^-$ leads to the "wrong way" downfield shift of aromatic protons.[8] This unusual chemical shift has previously been attributed to dimerization,[8] but a change in isomers and placing the proton on the N-atom in the Hdipic(NH)$^-$ isomer can also explain this observation. The spectra in Figure 3 show that the micelle affects the Hdipic$^-$ species which exist in distinctly different forms in aqueous and micellar environments.

The CTAB micellar interface stabilizes the nitrogen deprotonated monoanion Hdipic(N)$^-$, as indicated by only the small downfield shift of *para* proton (H_b) signal from pH 1.5 to 2.5 (Figure 3). At pH above 3.2 the chemical shift for both H_b and H_a protons in the presence of 50 mM CTAB are upfield from that observed in aqueous solution. To further demonstrate the downfield chemical shift upon protonation of the N-atom we recorded spectra in very acidic solutions, Figure S1. In these strongly acidic

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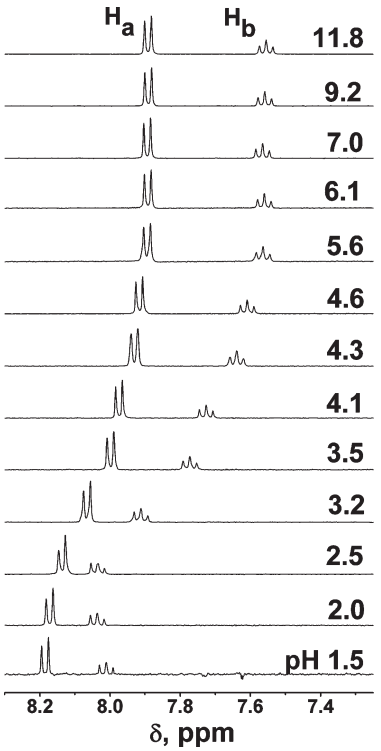

Figure 3. ^{1}H NMR spectra of 2 mM of H_2dipic in 50 mM CTAB in H_2O as shown at varying pH values. The measured solution pH is shown for each spectrum.

solutions, the nitrogen protonated H_3dipic$^+$ species showed the para (H_b) proton shifting more downfield than the two meta (H_a) protons (Figure S1). We conclude, that dipic monoanion in the CTAB micellar environment exists as the Hdipic(N)$^-$ isomer, in contrast to aqueous solution where the zwitterionic isomer Hdipic(NH)$^-$ is predominant, probably stabilized by inter- and intramolecular hydrogen bonding.

To provide additional evidence that micelles stabilize the nitrogen deprotonated species, we undertook a UV−vis spectroscopic study in both aqueous and CTAB micellar solutions. Specifically, the spectra of 0.1 mM dipic were recorded in 4 mM CTAB (pH from 1.56 to 7.05 (Figure S2b)) and in aqueous 30 mM NaCl solutions (Figure S2a). In the CTAB micelles the maximal molar absorptivity ε increases from ~3800 at pH 1.56 to ~4950 M^{-1} cm^{-1} at pH 2.51, and subsequently decreases to ~3500 M^{-1} cm^{-1} at pH 7.05 (Figure S2b). In aqueous dipic solution the maximal molar absorptivity is significantly higher, and it increases from ~4300 at pH 1.74 to ~6500 M^{-1} cm^{-1} at pH 3.52, and subsequently decreases to ~4200 M^{-1} cm^{-1} at pH 6.00 (Figure S2a). The UV absorbance of pyridine and pyridine-containing molecules is largely determined by the $\pi-\pi^*$ transition in the pyridine ring, and the molar absorptivity varies greatly with solution pH, which is usually attributed to the nitrogen protonation.[43−46] Comparison of UV spectra of 2-picolinic acid,[46] with its methyl ester and *N*-methyl betaine,[43] shows that nitrogen protonation has a major effect on molar absorptivity, whereas the carboxylic group protonation or deprotonation has no effect on the molar absorptivity. Only a minor red shift is observed upon carboxylate deprotonation in the methyl betaine of 2-picolinic acid.[43] The significant increase of ε (Figure S2a) upon H_2dipic (predominant species at pH 1.74) deprotonation to Hdipic$^-$ (pH 3.52) strongly supports our conclusion that pyridine nitrogen is protonated in aqueous Hdipic$^-$ solution. Further increase of solution pH reduces the molar absorptivity due to dianion dipic^{2-} formation (pH 6.00). These data suggest, that the nitrogen protonation is significantly reduced in the micellar solution, and that Hdipic$^-$ species absorbance is strongly modified by the CTAB micelles. The interpretation presented above regarding the different isomers of dipic in CTAB micelles based on the ^{1}H NMR studies are supported by UV−vis spectroscopic studies.

The absorbance spectra of the ligand provides information on the isomer distribution. In the case of 2,6-pyridine dicarboxylic acid both cationic H_3L^+ (ε = 9350) and monoanionic HL^- (ε = 6900) forms have higher absorbance than neutral H_2L (ε = 4890) and dianionic species (ε = 4500).[29] Interestingly, the pH-variable UV−vis spectroscopic study of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-pyridine dicarboxylic substituted acids[29] showed that molar absorptivity decreases upon increase of solution pH ($\varepsilon(H_3L^+) > \varepsilon(H_2L) > \varepsilon(HL^-) > \varepsilon(L^{2-})$) for all derivatives, except 2,6-substituted diacid (Figure S3). This indicates, that in the monoanionic 2,6-isomer the nitrogen is more protonated than the neutral species. Nitrogen protonation is confirmed by the high absorbance of protonated H_3L^+ species, which is observed for all pyridine dicarboxylic acids. This unique ability of the 2,6-substituted diacid to form the nitrogen-protonated Hdipic(NH)$^-$ monoanion is observed in crystal structures where the proton is coordinated to the nitrogen (0.92 Å) and two carboxylate oxygens (2.20 Å).[47] In conclusion, both ^{1}H NMR and UV−vis spectroscopies suggest, that Hdipic$^-$ in aqueous solution exists predominately in the nitrogen-protonated isomer Hdipic(NH)$^-$, whereas neutral molecule H_2dipic prefers the nitrogen-deprotonated isomer H_2dipic(N). In the CTAB micellar solutions, nitrogen deprotonated anion isomer Hdipic(N)$^-$ is the predominant species.

Solute pK_a is usually affected both by the surfactant nonpolar environment (medium effect) and by the charged interface (electrostatic effect).[31] The experimental evidence[31] shows that positively charged interface perturbs acid−base equilibrium stabilizing the anionic form of weak acids and thus reducing their pK_a values.[48] On the basis of the ^{1}H NMR data shown in Figure 3 in the presence of CTAB, we estimated two dipic pK_a values to be 2.0 ± 0.2 and 3.4 ± 0.2. The first pK_a value in aqueous solution is 2.2 whereas the second pK_a value is between 4.5[49] to 5.2,[29] demonstrating that the effect of CTAB is greater on the second pK_a value. Our observation is consistent with the CTAB interface is interacting stronger with dipic^{2-} than with the Hdipic$^-$, since pK_{a1} is not noticeably perturbed by the interface, whereas pK_{a2} is significantly reduced.

How CTAB Micelle Formation Affects Dipic^{2-} and HDipic$^-$. To test the possibility of whether the CTAB interaction with 2,6-pyridine dicarboxylic acid anion is not a simple ion pairing effect between the positively charged surfactant and relatively large hydrophobic anion compared to a micellar effect, we studied this system by ^{1}H NMR spectroscopy above and below CTAB cmc. In Figure 4, we show the CTAB concentration effect on the dipic^{2-} dianion chemical shift (pH 7.0). CTAB aggregates into spherical micelles above cmc of 0.8−1.1 mM.[40] Below 1 mM CTAB surfactant molecules exist mainly in disperse

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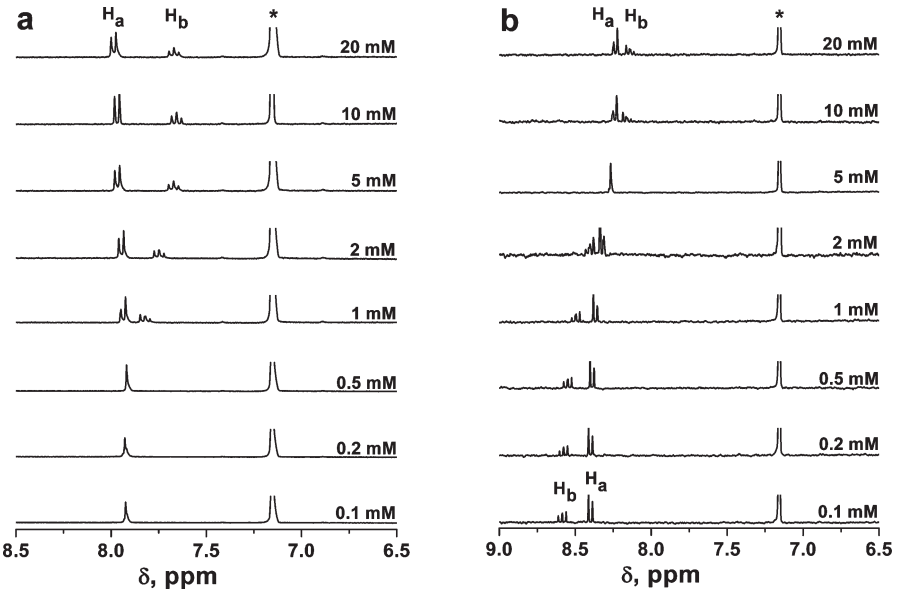

Figure 4. ^{1}H NMR spectra of 2 mM dipic^{2-} (a) and Hdipic$^-$ (b) as a function of the indicated CTAB concentration at pH 7.0 ± 0.5 (a) and pH 3.2 ± 0.1 (b). Spectra were taken at 35 °C with the lock on d_6-benzene in the coaxial capillary. The spectra were referenced against TMS added to the d_6-benzene. The singlet marked by an asterisk at 7.16 ppm is d_6-benzene.

form and at low surfactant concentration the dipic^{2-} spectrum is identical to that in aqueous solution (a singlet of overlapping protons H_a and H_b at 7.93 ppm), Figure 4. Increasing the CTAB concentration above 1 mM, the doublet H_a shifts downfield, and H_b shifts upfield as compared to aqueous solution. Upon further increase in CTAB concentration, the triplet H_b shifts upfield even more, reaching a constant value of 7.68 ppm at 5 mM surfactant concentration. The concentration dependent change in the spectra shows that dipic^{2-} anion is interacting with the micellar interface. Furthermore, the data in Table S1 show that cations, such as Na^+ and NMe_4^+, that form micelles are not associating with dipic^{2-}, because ^{1}H chemical shifts are concentration independent, and are the same in aqueous solution for both cations. These results suggest that the observed effects are due to the interactions with the micelle and not with a single CTAB molecule in an ion pair.

As have been shown previously for chloro-,[33] fluoro-,[32] and hydroxy[50,51]-benzoic acid derivatives, upfield shifts indicate the proton location inside the palisade layer (hydrated shell between the inner central core and the polar heads) of the surfactant micelle, whereas downfield-shifted proton signal suggests its location inside the Stern layer (surfactant heads and bound counterions) of the surfactant micelle. In our case, significant upfield shift of H_b proton indicates that dipic^{2-} anion moves from polar aqueous environment to relatively nonpolar hydrocarbon-like environment, and the slight downfield shift of two H_a protons is indicative of a more polar medium on the micellar interface. The pH study (Figure 3) shows that the dipic^{2-} at neutral pH remains in its dianionic form upon interaction with the micelle, therefore the proton chemical shift changes can be explained solely by the medium-induced effects, and not the dipic^{2-} protonation. Our results show that under the conditions investigated, the dipic^{2-} probe prefers to be associated with the micellar interface, even though this probe is readily soluble in aqueous solution at high concentration.

The effect of CTAB on Hdipic$^-$ protonation is related to the pK_a lowering from 5.2 in aqueous to 3.4 in CTAB micellar solution. At pH 3.2 in the experiments shown in Figure 4, the Hdipic$^-$ is the predominant species in aqueous solution, whereas in the CTAB micellar solution Hdipic$^-$ and dipic^{2-} amounts are almost equal. The deprotonation affects ^{1}H NMR chemical shifts as predicted, and upfield shift is observed.[8]

Although the downfield shift may be due to probe proton location inside the Stern layer, identifying the dipic location on CTAB micellar interface solely based on its ^{1}H chemical shift can be ambiguous because chemical shifts can be due to probe protonation. However, the ^{1}H chemical shifts of the CTAB protons upon micelle formation also provide valuable information on the possible probe location and extent of the probe and interface interaction. Furthermore, a control study in the absence of the probe provides benchmark data for the CTAB aggregation effect on the chemical shift. In Figure 5a (pH 7.0) and 5b (pH 3.2) we show the chemical shifts of CTAB protons (see Figure S4 for proton assignment) as a function of CTAB concentration in the presence of dipic-probe, and in the Figure 5c in solution (pH 7.2) with no probe. To quantify the effect on the proton chemical shielding, we calculated the micelle induced chemical shift as the difference $\Delta\delta = \delta_{20\text{mMCTAB}} - \delta_{0.1\text{mMCTAB}}$ in 20 mM CTAB solution and in 0.1 mM CTAB solution (below cmc). As expected, at both pH values of 3.2 and 7.0, the difference is negligible for deeply buried micellar carbon-chain methylene protons shift (from C_γ to C_o) and the terminal methyl proton. The nitrogen methyl protons located at the micellar−aqueous interface are slightly affected at both pH's ($\Delta\delta$ = 0.08−0.09 ppm), suggesting that dipic interaction with them (if any) are equally weak. The $C_\beta H_2$ protons and the $C_\alpha H_2$ shift strongly upfield upon micelle formation, suggesting that these methylene protons near the headgroup are interacting with the dipic. At pH 7.0, where dipic exists as a dipic^{2-} dianion, the $C_\beta H_2$ are shifted the most, compared to the $C_\alpha H_2$ protons. At pH 3.2 (Figure 5b), however, $\Delta\delta$ values for CTAB chemical shifts are indistinguishable from those observed in CTAB micelles with no probe (Figure 5c). This suggests, that monoprotonated dipic has very little impact on the CTAB, and it is interacting with the CTAB interface mostly electrostatically. The dianionic dipic^{2-} form, however, is penetrating

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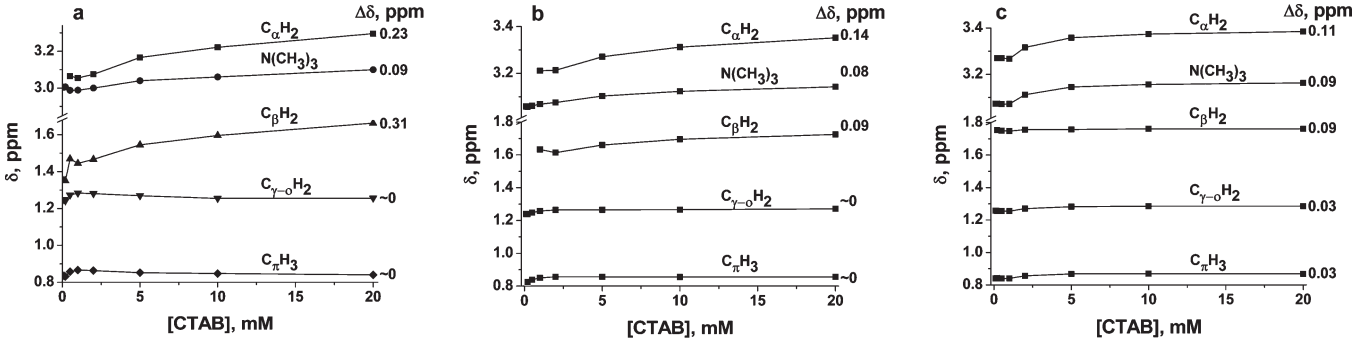

Figure 5. ^{1}H NMR chemical shifts of CTAB as a function of CTAB concentration at pH 7.0 ± 0.5 (a), 3.2 ± 0.1 (b), and 7.2 ± 0.6 (c). The dipic concentration in solution was 2 mM (a and b) and 0 mM (c). The magnitude of the micelle induced chemical shift difference Δδ is shown on the right.

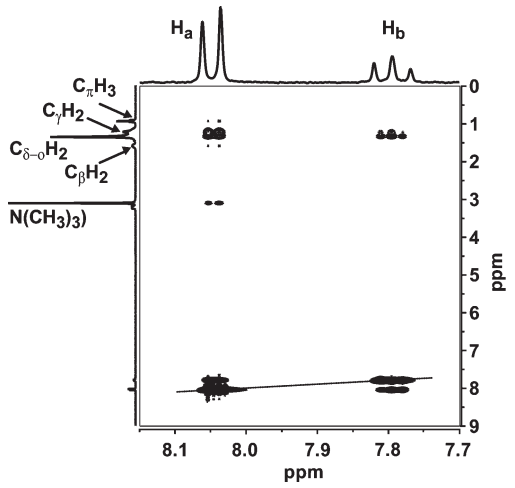

Figure 6. Excerpt of 2D ROESY spectrum showing dipic-CTA^+ cross peaks. Spectrum was acquired in 5 mM $dipic^{2-}$ and 10 mM CTAB solution in D_2O at pH 6.5 and 35 °C. Diagonal is indicated by a line.

deeper into the micellar interface. These observations are consistent with data reported on chlorobenzoates in cetyltrimethylammonium (CTA^+)[33−35] and in ($C_{22}N(CH_3)_3^+$)[52] micelles, and for fluorobenzoates in trimethyl(tetradecyl)ammonium (TTA^+) micelles.[32]

To further substantiate the assignments and penetration of the dipic ligand in the CTAB micelle, a 2D ROESY spectrum was recorded, Figure 6. The off-diagonal signals between the H_a and the $C_\beta H_2$, $C_{\delta-o}H_2$, $C_\gamma H_2$ and $C_\pi H_3$ and the $N(CH_3)_3$ signal and the H_b and the $C_{\delta-o}H_2$, $C_\gamma H_2$ suggest that the $dipic^{2-}$ has penetrated into the interface. This 2D spectrum confirms the interpretation of the 1D spectral shifts supporting that the probe is penetrating into the interface, but near the headgroup of CTAB. These studies demonstrate probe penetration and is consistent with the previous study showing similar penetration of chlorobenzoate into the CTA^+ interface.[33]

The observation that $dipic^{2-}$ penetrates the CTAB interface, but remains near the headgroup due to stabilization through Coulombic forces. Although the solubility of the $dipic^{2-}$ in aqueous solution is high, the ability of this compound to penetrate interfaces would allow the $dipic^{2-}$ to interact with both the charged headgroup and the hydrophobic alkyl chain. This location in the CTAB micelle is different than the location of $dipic^{2-}$ in AOT-isooctane reverse micelles, where the $dipic^{2-}$ is penetrating deeper into the interface.[8] Considering that AOT is negatively charged, the repulsion of the $dipic^{2-}$ molecules is reduced, resulting in deeper probe penetration. Studies with $[VO_2dipic]^-$ in AOT/isooctane reverse micelles also demonstrated deep penetration by the complex into the reverse micellar interface.[7] In general, changes in surfactant structure, counterion, temperature, and pH[53] are known to tune the micellar microstructure resulting in systems with varying properties. Although studies reported on these assemblies focus on their characterization, the importance of the location of the additives such as aromatic counterions near the micellar surface have been acknowledged.[53−55] Whether the difference in interface penetration observed in this study compared to our previous studies is due to the inherent differences between the micelle and the reverse micelle was tested by the following studies in CTAB reverse micelles.

Dipic in CTAB/1-Pentanol Reverse Micelles: Varying Water Pool pH. ^{1}H NMR spectra were recorded of 23 and 50 mM of aqueous dipic stock in CTAB/pentanol/cyclohexane reverse micelles prepared from stock solutions with pH ranging from 1.6 to 11.3, Figure 7.[56] The spectra shown in Figure 7 exhibit a splitting pattern that is different than that observed in aqueous solution and identical to that observed in the CTAB micelle, Figure 3. Both H_a and H_b signals shift upfield with increasing solution pH, which indicates that reverse micellar interface similar to micellar interface stabilize the nitrogen-deprotonated monoanion $Hdipic(N)^-$ isomer. Furthermore, a slight downfield shift is observed for H_b protons from pH 1.6 to 2.8 (Figure 7), similar to the CTAB micellar solutions (Figure 3). Although the chemical shifts are similar between the micelle and reverse micelle samples, they are not identical and thus present the evidence that some subtle differences exist between the two systems, Figure 8a. Both H_a and H_b resonances in reverse micelles are downfield from those observed in micelles, possibly suggesting that reverse micellar microenvironments are more polar than micelles and aqueous solution both.

The effect of interface headgroup charge on the CTAB ^{1}H chemical shift is illustrated further in Figure 8b, when comparing the reverse micellar systems based on cationic CTAB/pentanol/cyclohexane with reverse micelles based on anionic AOT/isooctane. This comparison shows that the dipic H_a proton environment in the CTAB reverse micelle is similar to the environment of both the H_a and H_b protons in the AOT reverse micelle.[8] The H_b proton

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(56) The possibility that the amount of pentanol in cyclohexane changed the polarity of cyclohexane solvent and thus significantly increased the solubility of Na_2dipic was ruled out, because no dipic resonances were detected by ^{1}H NMR spectroscopy of pentanol−cyclohexane solutions saturated with solid Na_2dipic.

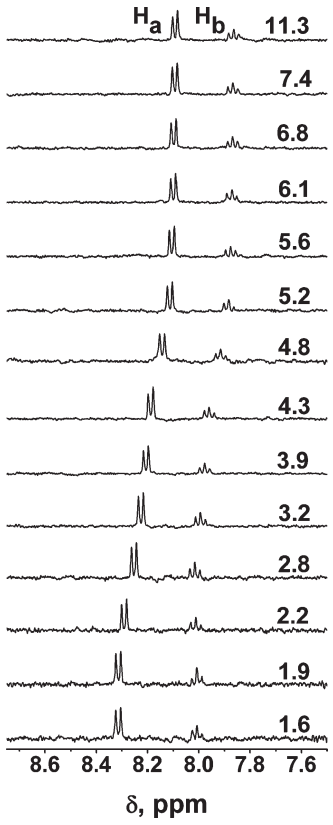

Figure 7. ^{1}H NMR spectra of 50 mM dipic at pH from 3.2 to 11.3 and 23 mM dipic at pH from 1.6 to 2.8 in reverse micelles prepared in cyclohexane/0.15 M CTAB, 0.75 M 1-pentanol; w_0 = 6. Aqueous dipic stock solution pH is shown next to each spectrum. Spectra were recorded at 24 °C, chemical shifts were referenced against the cyclohexane resonance at 1.443 ppm.

environment in CTAB is probably more hydrophobic considering the further upfield shifts of the dipic protons in the AOT.

Dipic in CTAB Reverse Micelles: Varying the Water Pool Size. To illustrate the effect of the nanodroplet size on solute interactions in the reverse micelles, we show in Figure 9a the spectra for dipic solutions at pH 6.6 at low CTAB concentrations where the spectrum is identical to that in aqueous solution and then at 20 mM CTAB in the presence of micelles, as well as in CTAB/pentanol reverse micelles of sizes w_0 = 6 and 20. These spectra show that at pH 6.6, the water pool size has little effect on the chemical shift of dipic^{2-} protons in CTAB reverse micelles, illustrating that probe molecule location remains the same and that dipic^{2-} environments are different in CTAB micelles and reverse micelles. Comparing spectra in aqueous solution with both micellar and reverse micellar systems, H_a proton signal shifts downfield and the H_b shifts upfield, indicating similarities in the interface effect on the probe location. However, in micellar solution H_b proton is affected significantly more than in the reverse micelle compared to the aqueous solution, suggesting that dipic^{2-} is penetrating deeper into the micellar interface.

In contrast, at pH 3.2, dipic protonation state and location are similar in CTAB micelles and large (w_0 = 20) reverse micelle shown in Figure 9b. However, in the smaller water pool (w_0 = 6) both Hdipic$^-$ signals move upfield which indicates either additional deprotonation or stronger interaction with the interface or both of the above. This observation shows that CTAB micellar and reverse micellar interfaces are similar for Hdipic$^-$, but different for dipic^{2-}, which could imply that the dianion is interacting with the interface stronger than the monoanion. The splitting pattern at pH 6.6 suggests that the penetrating species remain dipic^{2-} and no protonation occurs upon penetration of the reverse micellar interface. The greater variation in shifts in the reverse micelle system at pH 3.2 documents the range of different environments that are created in these reverse micelles as the w_0 is changing. In the negatively charged AOT reverse micelles dipic^{2-} resonances shift significantly downfield with decreasing w_0,[57] indicating that probe location is sensitive to the water pool size. Since the Hdipic$^-$ monoanion and not the dianion is affected by decreasing the water pool, additional deprotonation could be causing the observed changes with w_0 decrease.

Comparison of Solute Studies in CTAB Micelles and Reverse Micelles. Figure 10 illustrates a possible location of dipic^{2-} in the CTAB micelle (left) and the CTAB reverse micelle (right). In the CTAB micelle, the data supports the location of dipic^{2-} to be in the Stern/palisade layer. Although differences exist in the CTAB reverse micelle system, the chemical shift patterns are consistent with a similar probe location in this system. Attempts to characterize the reverse micelle system using 2D NMR studies were not successful for the samples used in these studies. However, the greater upfield shifts of the dipic^{2-} in the CTAB reverse micelle compared to the AOT reverse micelles is consistent with an interpretation of a solute location nearer the charged interface. Undoubtedly, the presence of the alcohol impacts the nature of the interface[58] and thus how it interacts with solutes since 2−3 molecules of 1-pentanol are associated with each CTAB molecule. However, the previous studies by Palazzo and co-workers suggested that even large and charged solutes such as nucleotides do not in a major way affect the CTAB reverse micellar structures[59,60] or other reverse micellar structures,[10,11] when examining a fluorescent probe molecule. Some additives such as glycerol, formamide, ethylene glycol, and bile salts in formulations of reverse micelles could change the spherical micelles to worm-like micelles as confirmed using dynamic light scattering, rheological and small-angle diffraction data.[53] Such dramatic changes in microstructure are not expected with dipic as a solute.[8,57]

These studies presented here were designed for the self-assembled nanostructures, that is the micelles and the reverse micelles to be compared, were of similar size (Table S2). The interactions between dipic^{2-} and the micelle or reverse micellar interfaces, respectively, are dependent on the specific details of the assemblies. This is particularly obvious for the reverse micelle-systems where we see distinct differences in chemical shifts with the w_0 size of the reverse micelle. In addition, counterion and solvent effects are to be anticipated (Table S1). Specifically for these systems we have found that deprotonation causes upfield shifts, and this is observed both in aqueous (Figure S1) and in organic solvents (Table S1). The smaller the cation, the further downfield shift is observed. These observations are in agreement with known effects of protonation or cation association on chemical shifts.[42] Deviation from this trend was observed in aqueous solution for H_2dipic and was attributed to a dimerization of the zwitterions.[8] As we demonstrate in this work, upfield shift can also be observed with changes in isomers, and pyridine-ring protonation.

Developing the observations in this work into a general set of guidelines is straightforward for micelles, since similar patterns and changes in chemical shifts consistently showed upfield shifts

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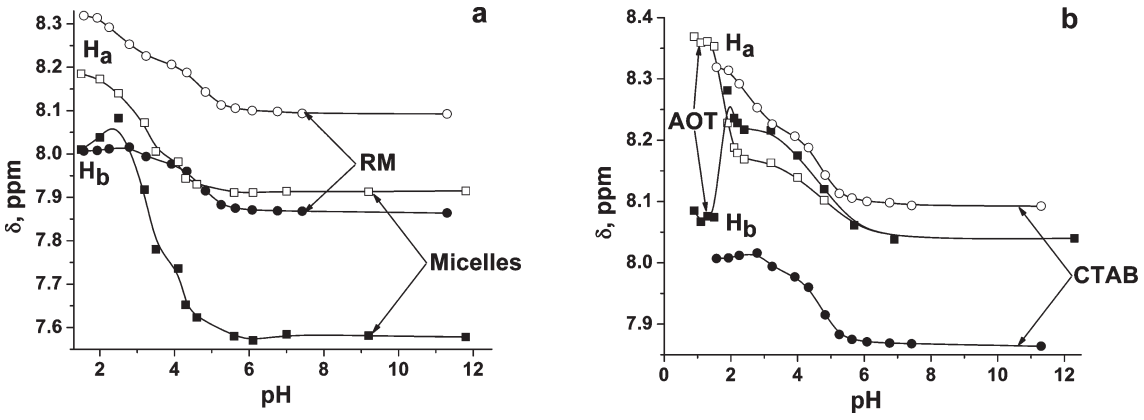

Figure 8. ^{1}H chemical shift of dipic protons is shown as a function of aqueous stock solution pH for CTAB micellar and reversed micellar solutions $w_0 = 6$ (a), and for CTAB reverse micellar $w_0 = 6$ and AOT reverse micellar solutions $w_0 = 6$ (b). Data points were taken from spectra shown in Figures 3 (CTAB micelles), 7 (CTAB reverse micelles), and ref 8. (AOT reverse micelles). Open symbols (H_a) and full symbols (H_b) show the chemical shifts.

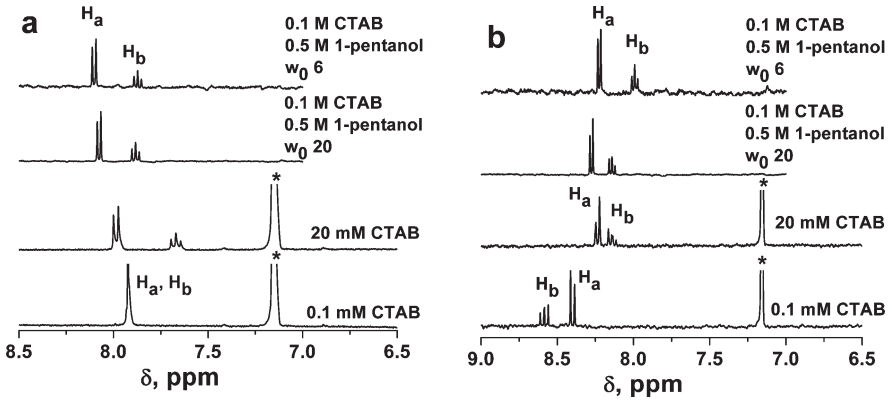

Figure 9. ^{1}H NMR spectra of dipic^{2-} at pH 6.6 (a) and Hdipic$^-$ at pH 3.2 (b) in aqueous solution (with disperse CTAB micelles), and CTAB/1-pentanol/cyclohexane reverse micelles.

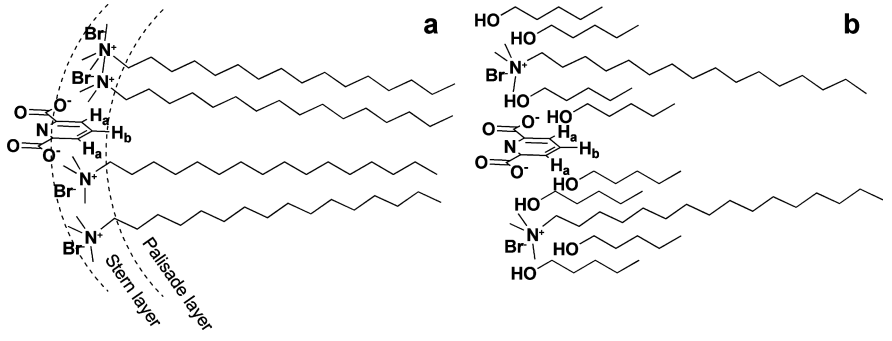

Figure 10. Possible dipic^{2-} location at the CTAB micellar (a) and reverse micellar (b) interface.

for solute penetration in previous work.[37] These patterns are confirmed with the dipic-probe in these studies. However, penetration of solutes in AOT reverse micelles are reported to be accompanied by downfield shifts.[8] In this work we demonstrated that this difference between AOT and CTAB reverse micelles is probably due to the differences in headgroup charge, and not to an inherent difference between micelles and reverse micelles as illustrated in Figures 8a and S5−S6. However, we do find that the dipic-probe protons in micelles generally appear somewhat upfield from dipic-probe in reverse micelles. This is attributed to the more hydrophobic and less polar environment deep in the interface layer of a micelle compared to the more dynamic interface layer of a reverse micelle that would contain penetrating pentanol and water.

The comparison with aqueous probes can be more difficult if the probe such as dipic has different protonation states and isomers. Thus, the chemical shifts for the dipic-probe range from 8.0 to 8.5 ppm, and at neutral pH the two different protons overlap. Interestingly, shifts in the two protons on the dipic-probe can be different upon placement in the micelle and reverse micelle. This presumably reflects a difference in location and interface interaction between the two protons. The surprising observation is that these protons sometimes shift upfield and other times downfield upon solute penetration beyond the polar headgroups into the interface. Our studies were designed to compare CTAB micelles and reverse micelles, and we find that penetration of micelles and reverse micellar interface is reflected by similar shifts.

That is, solute-proton location near the headgroup is reflected by a downfield shift and deeper penetration manifests itself by upfield chemical shifts associated with these CTAB interfaces. In contrast, dipic-probe has been observed penetrating deeply into AOT/isooctane reverse micelle interfaces while exhibiting a downfield chemical shift.[8] Since the shift is opposite to our observations with the dipic-probe in CTAB, we propose that the particular chemical shift may be associated with the charge of the solute and surfactant headgroups.

In summary, we have investigated in detail the environment of a pH-sensitive probe dipic^{2-} in microemulsions using ^{1}H NMR spectroscopy. Interpretation of 1D ^{1}H NMR chemical shifts is complex, but with careful consideration of the nature of the microemulsion under investigation as well as the charges of the specific systems, consistent patterns emerge depending on the location of the solute and the charge of the surfactant headgroup. We have characterized the environment of this spectroscopic probe in CTAB based microemulsions and find that dipic^{2-} is able to penetrate the surfactant interface, regardless of the charge of the headgroup, and whether the microemulsion is a micelle or a reverse micelle. The fact that dipic^{2-} also penetrates negatively charged interfaces such as those created with AOT suggest that the Coulombic forces alone are not sufficient to preclude penetration of a solute. It appears that the solute readily adjusts its location in the interface based on charge. Deeper penetration into the interface reduces the negative interactions of a solute with a similarly charged headgroup. These studies show that not only lipophilic compounds are effectively solvated by nanosized microemulsion assemblies and provide support to continue to identify applications of these systems in drug formulations including with both lipophilic and hydrophilic drugs.

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Supporting Information Available: ^{1}H NMR spectra of H_3dipic$^+$ cation in trifluoroacetic acid, pH-variable UV−vis spectra of dipic in aqueous NaCl and CTAB solutions, plots of dipic ^{1}H NMR chemical shifts as a function of pH in CTAB micellar and aqueous, CTAB reverse micellar and aqueous solutions, ^{1}H NMR chemical shifts of dipic^{2-} and H_2dipic in various media. This material is available free of charge via the Internet at http://pubs.acs.org.