"Snap-Shooting" the Interface of AOT Reverse Micelles: Use of Chemical Trapping

Gollapudi Venkata Srilakshmi and Arabinda Chaudhuri*[a]

Abstract: The first use of the phenyl cation trapping technique in "snap-shooting" the local molar concentrations of water and sulfosuccinate head-groups in the interfacial region of AOT–2,2,4-trimethylpentane–water reverse micelles has been accomplished. Our results demonstrate that the interfacial concentrations of the sulfosuccinate head-groups in AOT (0.1 M)–2,2,4-trimethylpentane–water reverse micelles are remarkably high (2.75–2.34 M) across the W_0 (the molar ratio of water to surfactant) range 12 to 44. However, the interfacial concentrations of water in AOT–2,2,4-trimethylpentane–water reverse micelles across the same range of solution compositions are significantly lower (27.9–32.0 M) than the molar concentration of bulk water (55.5 M). The present results provide new insight on the microenvironments of interfacially located enzymes such as lipases entrapped in AOT–2,2,4-trimethylpentane–water reverse micelles, the most extensively exploited reverse-micellar system in micellar biotechnology.

Keywords: chemical trapping · diazo compounds · interfacial concentrations · micelles

Introduction

Reverse micelles (also known as water-in-oil microemulsions and reverse swollen micelles) are optically transparent nanometer-scale droplets of water in a bulk apolar organic solvent formed with the help of aggregated surfactant and cosurfactant molecules at the organic–water interface.[1–3] The polar aqueous core of such reverse-micellar aggregates is popularly known as the water-pool (Figure 1). Technological and biotechnological potentials of reverse micelles have already been amply demonstrated. Proteins, enzymes, and nucleic acids have been solubilized in the reverse-micellar water-pools without the loss of their biological activities.[4–10] Reverse micelles are also exploited in the synthesis of technologically important inorganic materials of submicron dimensions (nanoparticles).[11–18]

Despite the above-mentioned widespread applications of reverse micelles, several microstructural parameters of reverse-micellar aggregates are still incompletely understood. For example, a number of investigations have been reported on the encapsulation of lipase in the sodium bis(2-ethyl-1-hexyl)sulfosuccinate (AOT) anionic reverse micelle without the loss of its biological activity.[1, 2, 3, 19, 20] Lipase, an interfacially active enzyme,[21] is likely to locate itself in the anisotropic interfacial regions of the anionic AOT reverse micelle. Thus, the local molar concentrations of the molecules and ions present in the interfacial regions of AOT reverse micelles are expected to play a key role in the modulation of the activities of such an interfacially encapsulated lipase. However, direct experimental estimations of the local molar concentrations of water and the sulfosuccinate head-groups in the interfacial region of AOT reverse micelles have never been reported.

Interfacial concentrations in aqueous cationic micelles and microemulsions can be measured by a phenyl cation trapping method developed by Romsted and co-workers.[22–24] In this method, the product yields from dediazoniation of 2,6-dimethyl-4-*n*-hexadecylbenzenediazonium tetrafluoroborate (16-ArN_2^+, a surfactant-like hydrophobic arenediazonium salt used as the interfacial probe) bound to cationic cetyltrimethylammonium halide (CTAX) micelles are assumed to be proportional to the concentrations of nucleophiles at the cationic-micellar interface. Recently, we have found a novel use of 16-ArN_2^+ for sensing the interfacial concentrations of hydroxide ions at the *exo* surfaces of various cationic liposomal gene-delivery reagents.[25] Our new method[25] is potentially applicable for sensing the *exo* surface pH values of a wide range of cationic liposomal systems[26] such as the novel nonglycerol-based monocationic transfection lipids

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Supporting information (HPLC peak areas, observed and normalized product yields in reverse-micellar and nonmicellar aqueous dediazoniation reactions and all the HPLC calibration equations used (Tables S1-S2); pseudo first-order kinetics plots for both the nonmicellar and AOT reverse-micelle bound dediazoniation reactions and the ^1H NMR (200 MHz) spectra of 1-ArS, 16-ArS and SDSS) for this article is available on the WWW under http://www.wiley-vch.de/home/chemistry/ or from the author.

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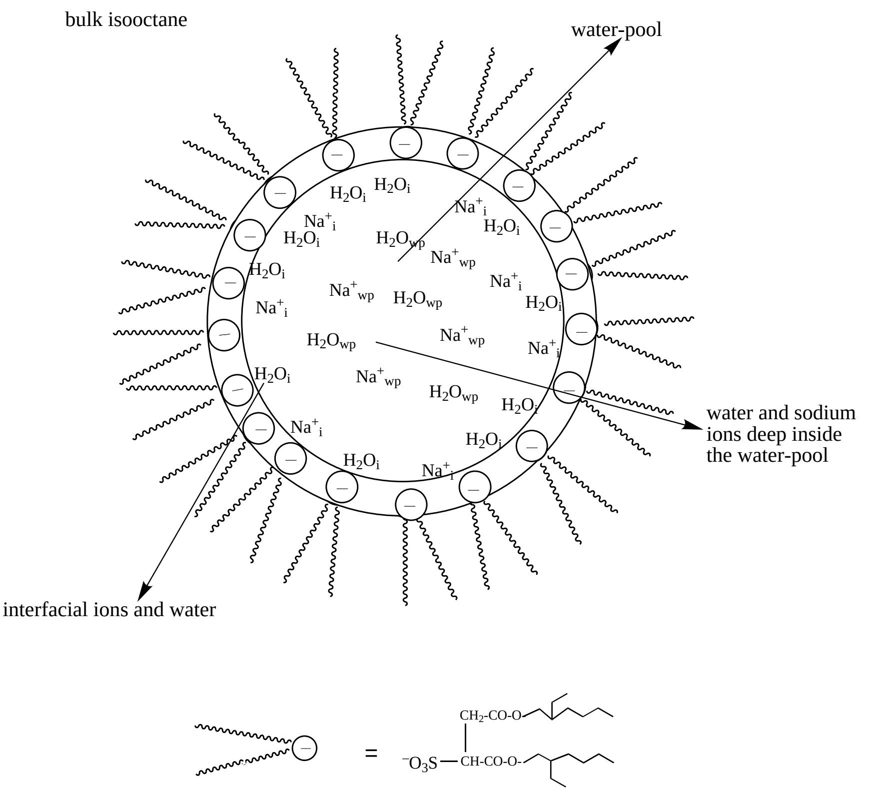


Figure 1. Schematic representation of an anionic reverse-micellar system. The anionic head groups of the surfactant are located in the polar interfacial region. The subscripts i and wp represent the interfacial and the inside water-pool regions respectively. The interfacial boundary drawn in solid circles is purely schematic (interfacial layers of reverse-micellar aggregates are, in reality, highly flexible and dynamic in nature).

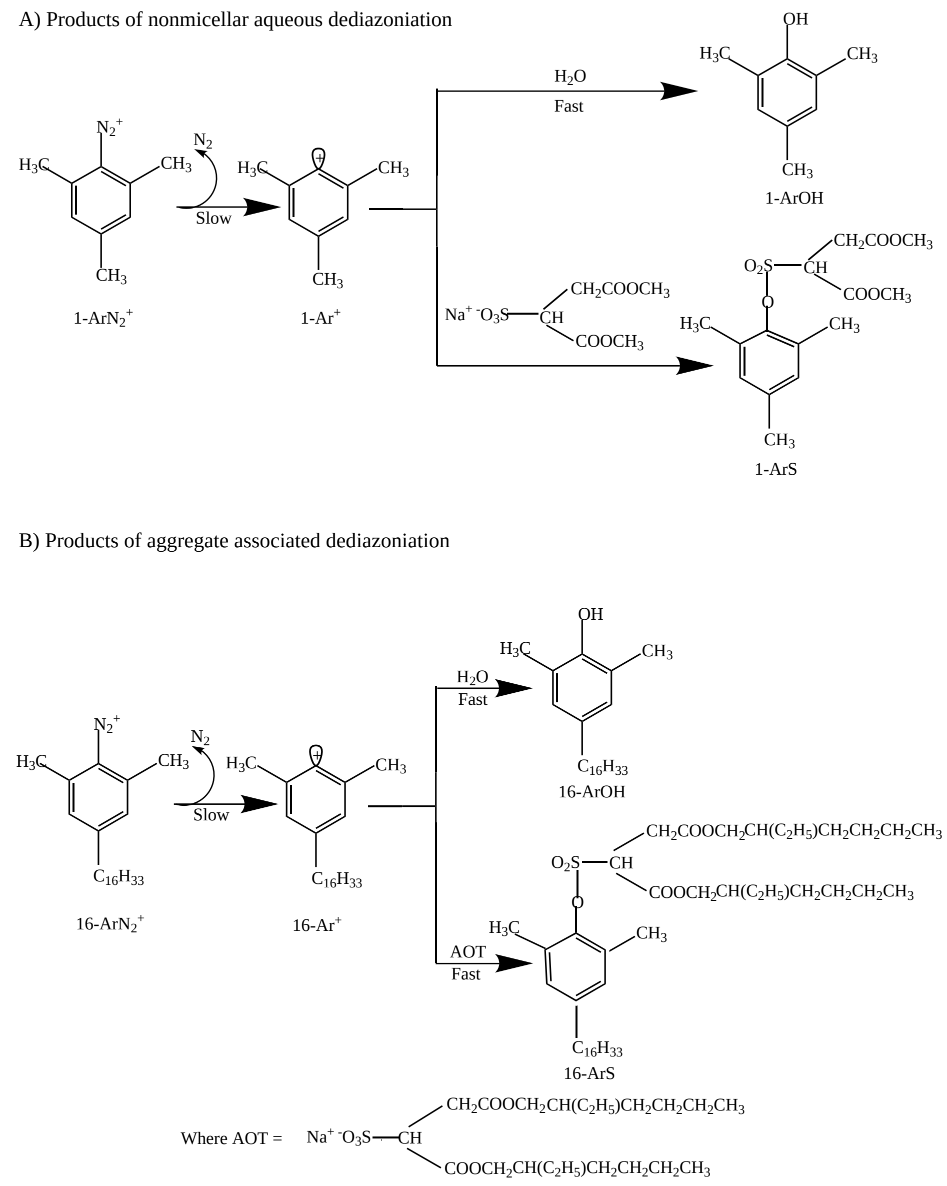
recently developed by us.[27] We have also extended the phenyl cation trapping method to the estimation of the interfacial concentrations of cationic reverse-micellar aggregates.[28–30] However, the unavailability of the AOT head-group mimic, nonmicelle-forming, water-soluble sodium sulfosuccinate salt, and the lack of information about the identities of the dediazoniation products have so far precluded the use of chemical trapping in the estimation of the interfacial compositions of anionic AOT reverse micelles, one of the most extensively exploited reverse-micellar systems in micellar biotechnology. In the present work, we have determined the structural identities of the dediazoniation products formed in the AOT–2,2,4-trimethylpentane–water reverse-micelle associated dediazoniation of 16-ArN_2^+, and have prepared the hitherto unknown nonmicelle-forming, water-soluble sodium sulfosuccinate salt. Herein, we delineate the first use of chemical trapping in "snap-shooting" the interfacial compositions of anionic AOT–2,2,4-trimethylpentane–water reverse micelles across a wide range of solution compositions.

Results and Discussion

Method of "snap-shooting" AOT reverse-micelle interface: The surfactant-like amphiphilic arene diazonium salt 2,6-dimethyl-4-hexadecylbenzenediazonium tetrafluoroborate (16-ArN_2^+, Scheme 1B) is allowed to undergo a thermal dediazoniation reaction in the anionic AOT–2,2,4-trimethylpentane–water reverse micelle at ambient temperature in the dark across a wide range of W_0 (the molar ratio of water to AOT). The reactive polar diazonio group of 16-ArN_2^+ locates itself in the interfacial region anchoring its hydrophobic *n*-hexadecyl tail into the bulk 2,2,4-trimethylpentane. Because of its surfactant-like structure and its low concentration (less than 1 mol% with respect to surfactant), 16-ArN_2^+ causes minimum perturbation of the aggregate structures. The arenediazonio group of 16-ArN_2^+ reacts with the available interfacial nucleophiles, namely, water and the anionic sulfosuccinate head-groups of AOT, and is quantitatively converted into the products 16-ArOH and 16-ArS (Scheme 1B). Following the primary logic of the chemical-trapping approach,[22, 23] the nonmicellar aqueous dediazoniation reaction of 1-ArN_2^+ in the presence of varying amounts of AOT head-group mimic sodium dimethylsulfosuccinate, SDSS (Scheme 1A) is assumed to be a good model for the AOT reverse-micelle interface-bound dediazoniation of 16-ArN_2^+ (Scheme 1B). In other words, the correlation between the percentage yields of 1-ArOH and the concentration of H_2O (M) observed for nonmicellar aqueous dediazoniations of 1-ArN_2^+ in the presence of varying amounts of H_2O (M) (Figure 2A) is assumed to be the same as that between the percentage yields of 16-ArOH and the interfacial concentrations of water, $[H_2O_i]$ (M) in AOT reverse-micelle bound dediazoniation of 16-ArN_2^+. Thus, $[H_2O_i]$ values (Table 1) were estimated by substituting the 16-ArOH percentage yields (Table 1) produced in AOT reverse-micelle bound dediazoniation of 16-ArN_2^+ in Equation (1).

$$\%\,16\text{-ArOH} = 0.914[H_2O_i](\text{M}) + 49.72 \qquad (1)$$

Similarly, the correlation between the percentage yields of 1-ArS and the concentration of SDSS (M) observed during nonmicellar aqueous dediazoniations of 1-ArN_2^+ in the presence of varying amounts of [SDSS] (M) (Figure 2B) is assumed to be the same as that between the percentage yields of 16-ArS yields and the local molar concentrations of sulfosuccinate head-groups in the interfacial region, $[SS_i]$ (M), in AOT reverse-micelle bound dediazoniation of 16-ArN_2^+. Thus, $[SS_i]$ (M) values shown in Table 1 were estimated by substituting the percentage yields of 16-ArS (Table 1)



Scheme 1.

produced in AOT reverse-micelle bound dediazoniation of 16-ArN_2^+ in Equation (2).

$$\%16\text{-ArS} = 9.036[SS_i](\text{M}) - 0.103 \qquad (2)$$

The primary assumption used in the present investigation to "snap-shot" the interfacial concentrations of water and sulfosuccinate head groups in AOT reverse micelles is that the aqueous nonmicellar dediazoniation product distribution (Scheme 1 A) is a good model for the product distribution in the AOT interface-bound dediazoniation (Scheme 1 B).[22, 23] Thus, the local molarities of the sulfosuccinate head-groups in the interfacial region of AOT reverse micelles, $[SS_i]$ (M), across the range $W_0 = 12-44$ (Table 1) were determined by substituting the yields of 16-ArS in AOT reverse-micelle interface-bound dediazoniation of 16-ArN_2^+ (Table 1) in the correlation % 16-ArS = $9.036[SS_i] - 0.103$, correlation coefficient 0.997 (the same correlation between % 1-ArS and [SDSS](M) for the nonmicellar thermal dediazoniation of 1-ArN_2^+ across a range of added SDSS is shown in Figure 2B). The same logic justifies the validity of using Equation (1) to estimate the interfacial concentrations of water ($[H_2O_i]$ (M), Table 1) in AOT reverse micelles across the W_0 range 12–44. The word "snap-shooting" in the present investigation has been used in a purely qualitative sense. The measured interfacial concentrations are concentrations of the time-independent equilibrium states and are not pictures taken at a certain time specific for that measurement.[31]

The extraordinary insensitivities of dediazoniation reactions to medium effects[32–34] justify the validity of using the nonmicellar aqueous dediazoniations of 1-ArN_2^+ as the model for aggregate-associated dediazoniations of 16-ArN_2^+. In order to get a quantitative sense of the degree of medium effects, if any, for the dediazoniation reactions in the presence of sulfosuccinate nucleophiles (Scheme 1), we have determined the pseudo-first-order room-temperature dediazoniation rate constants for both nonmicellar aqueous dediazoniation of 1-ArN_2^+ at pH 7.0 in the presence of 0.5 M SDSS and AOT reverse-micelle associated dediazoniation of 16-ArN_2^+. The observed dediazoniation rate constants are $9.00 \times 10^{-2}\,h^{-1}$ for 1-ArN_2^+ and $5.10 \times 10^{-2}\,h^{-1}$ for 16-ArN_2^+ (the linear first-order kinetic plots are provided in the Supporting Information). These kinetic results are consistent with our assumption of minimal medium effects in arenedediazoniation reactions.

Another inherent assumption of the present method is that the reaction rates of the intermediate phenyl cations (1-Ar^+ and 16-Ar^+, Scheme 1) with the neighbouring nucleophiles are much faster than the diffusion rates of the nucleophiles in the reverse micelles. This assumption is supported by the known characteristics of arenediazonium ions. In aqueous acid in the dark, arenediazonium ions lose N_2 spontaneously to give a very reactive aryl cation[32–34] (Scheme 1). The extraordinary insensitivities of dediazoniation reactions to medium effects[30, 32, 33] and their remarkably low selectivities toward weakly basic anionic and neutral nucleophiles[23, 24, 32–34] are consistent with the formation of highly

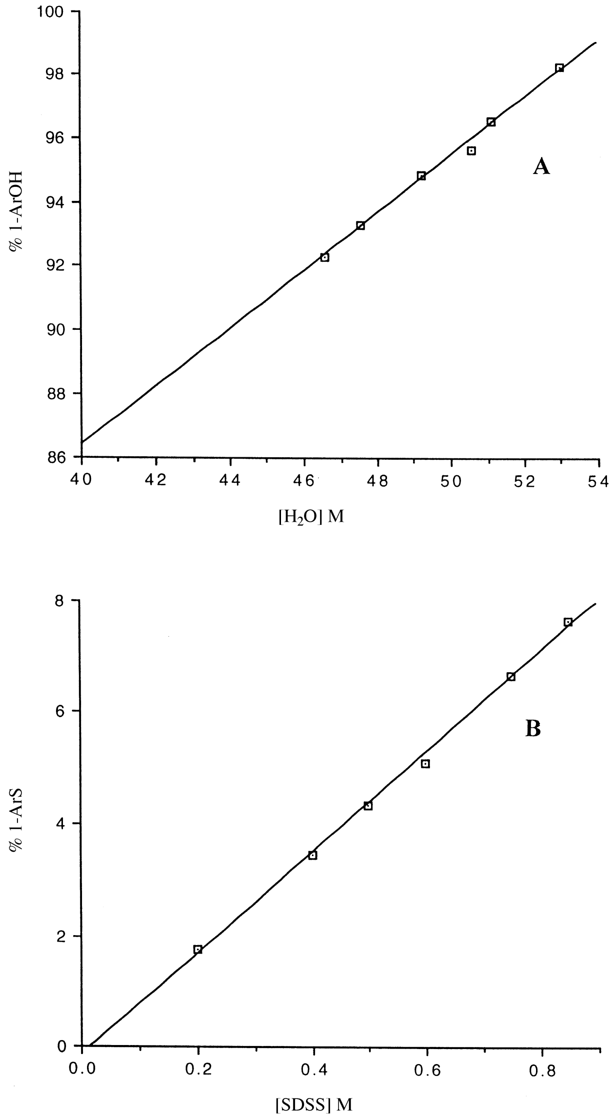


Figure 2. A) Yields of 1-ArOH in the nonmicellar aqueous dediazoniation reaction of 1-ArN_2^+ across the added range of water concentrations. The curve was fitted by the equation $\%1\text{-ArOH} = 0.914[H_2O]$ (M)$+49.72$ correlation coefficient $= 0.990$. B) Yields of 1-ArS in the nonmicellar aqueous dediazoniation reaction of 1-ArN_2^+ across the added range of AOT head-group mimic water-soluble SDSS. The curve was fitted by the equation $\%1\text{-ArS} = 9.036[\text{SDSS}]$(M) $- 0.103$ correlation coefficient $= 0.997$.

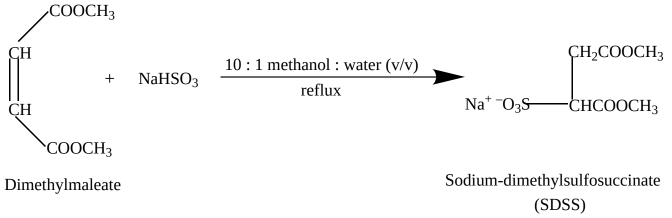
reactive phenyl cation intermediates with a very short life time.[32, 33, 35–37] The charge distributions in the arenediazonium ion and in aryl cations are very similar.[38] Thus, reorganization of molecules and ions within the immediate vicinity of the diazonio groups as a result of diffusion of nucleophiles in reverse micelles should be minimal during the transition from the aryl cation to the products (Scheme 1), and the overall product distribution reflects the equilibrium distribution of ions and molecules around the ensemble of diazonio groups in their ground states.

The chemical structures of both the new dediazoniation products, namely, 1-ArS and 16-ArS (Scheme 1) were confirmed by their actual isolation followed by spectroscopic structure elucidations of the purified materials. Both the nonmicellar aqueous dediazoniation of 1-ArN_2^+ and the reverse-micelle bound dediazoniation reactions of 16-ArN_2^+ were carried out on relatively much larger scales during the actual isolation of the dediazoniation products 1-ArS and 16-ArS to ensure isolation of sufficient amounts of purified 1-ArS and 16-ArS. As detailed in the Experimental Section, the AOT head-group mimic salt SDSS (Scheme 2) was synthesized in a single step by reacting dimethyl maleate with sodium bisulfite in methanol under refluxing conditions.

Table 1. Normalized product yields[a] in AOT–2,2,4-trimethylpentane–water reverse micelle bound dediazoniation of 16-ArN_2^+ and the estimated interfacial concentrations.

	Normalized yields			
W_0	16-ArOH [%]	16-ArS [%]	$[H_2O_i]$ [M]	$[SS_i]$ [M]
12	75.3	24.7	27.92	2.75
16	77.0	23.0	29.85	2.55
20	77.7	22.3	30.55	2.48
24	78.1	21.9	30.98	2.44
28	78.5	21.5	31.50	2.38
32	79.2	20.8	32.28	2.31
36	79.4	20.6	32.46	2.29
40	79.0	21.0	32.06	2.33
44	78.9	21.1	31.95	2.34

[a] Because quantitative conversions to expected dediazoniation products were always observed (actual yields of 16-ArOH and 16-ArS in duplicate separate experiments varied within ±1%) with no significant extaneous peaks in the HPLC chromatograms, normalized product yields were used in estimating the interfacial concentrations. The actual observed yields, HPLC peak areas and the HPLC calibration curves used in determining the product yields are available in the Supporting Information (Table S2).



Scheme 2. Synthesis of SDSS.

In the construction of the percentage yield 1-ArS versus [SDSS] (M) correlation (Figure 2B, the calibration graph used for the estimation of the interfacial concentrations of the sulfosuccinate head-group in AOT reverse micelles), we have used 0.85 M as the maximum concentration of SDSS (the solubility limit of SDSS in water is around 1.0 M). The molar concentrations of water used in constructing the %1-ArOH yields versus $[H_2O]$ (M) correlation (Figure 2A, the calibration graph used for the estimation of the interfacial concentrations of water in AOT reverse micelle) was in the range 53.0–46.6 M. The water and SDSS concentrations used in constructing the correlations shown in Figures 2A and 2B were determined from the total solution volume and the exact weight of water and SDSS. Notably, the volume occupied by the sulfosuccinate groups themselves are remarkably high since the concentration of water in 0.85 M aqueous SDSS solution is 46.6 M (Table 1), a value that is significantly lower than the concentration of bulk water in absence of any dissolved SDSS (55.5 M).

Our results (Table 1) show that the interfacial region of AOT reverse micelles is densely populated by the sulfosuccinate head-groups and the local molar concentrations of water in the interfacial region of AOT reverse micelles are significantly less than the molar concentrations of bulk water (55.5 M). The values of [SS_i] across the W_0 range 12–44 are in the range of 2.75–2.34 M and the corresponding range for [H_2O_i] is 27.9–32.0 M (Table 1). Thus, the measured interfacial concentration range of the sulfosuccinate head-groups of AOT are significantly higher than the molar concentration range of the AOT head-group mimic SDSS (limited solubility of SDSS in water did not permit experiments beyond 0.85 M SDSS) used in constructing the calibration graph (Figure 2B). In the measurement of the otherwise unattainably high interfacial concentrations of *n*-butanol (5.0–10.0 M) in *n*-butanol swollen aqueous cationic cetyltrimethylammonium bromide micelles, Romsted and his coworkers have also used similar nonmicellar aqueous dediazoniation reactions of 1-ArN_2^+ across a low range of added *n*-butanol (maximum solubility of *n*-butanol in water is around 1.0 M).[23] Extraordinary insensitivities of dediazoniation reactions to medium effects permits the use of such a calibration graph (Figure 2B). A suitable water-miscible solvent that will increase the solubility of SDSS and will not react with the reactive diazonio group can, in principle, be used in constructing calibration graphs to give higher and lower molar concentration ranges of SDSS and water.

Since, the interfacial concentrations of sulfosuccinate head-groups and water in AOT reverse micelles have not been experimentally determined before, an issue that comes naturally at this point of discussion is: "How reliable are these local interfacial concentrations of sulfosuccinate head-groups estimated by the present trapping method?" Since the reactive diazonio group of 16-ArN_2^+ probes the polar region of the AOT reverse-micelle interface, we assume that the polar region sensed by 16-ArN_2^+ is the sum of the reported thickness of the bound-water layer in AOT reverse micelles (5 Å)[39–42] and the length of the polar head-group region of AOT (that is, the length of the sulfosuccinate head-group plus the length of the ester group of AOT which can be taken as approximately equal to 5 Å). Thus, assuming a total interfacial thickness of 10 Å is probed by 16-ArN_2^+, taking 30 Å as the water-pool core radius of AOT reverse micelles[42] and using 302 as the mean average aggregation number at $W_0 = 20$,[43] we have made an independent estimation of the local molar concentration of sulfosuccinate head groups in AOT reverse micelles (using a spherical geometry for reverse micellar water-pool). Our independently estimated value of [SS_i] = 3.23 M at $W_0 = 20$ compares reasonably well with [SS_i] = 2.48 M at $W_0 = 20$ estimated by the present chemical-trapping method (Table 1). The ratio of [H_2O_i] to [SS_i] determined by the present trapping method varies from about 10 at $W_0 = 12$ to about 14 at $W_0 = 44$ (Table 1). These ratios are consistent with the hydration number 13 (that is, the number of water molecules bound per AOT molecule) for AOT–2,2,4-trimethylpentane–water reverse-micelle system obtained by Hauser et al. using deuterium magnetic resonance Spectroscopy (^{2}H NMR) of AOT reverse micelles prepared in D_2O.[44] Thus, the [H_2O_i] values estimated by the present method (Table 1) are sensible numbers. Our results demonstrate that the local molar concentrations of water and sulfosuccinate head-groups in AOT–2,2,4-trimethylpentane–water reverse micelles do not change significantly across $W_0 = 12–44$. Since both size and aggregation number of reverse micelles increase with increasing W_0, such grossly unchanged interfacial compositions are not unlikely at all. Romsted's trapping technique[23] can measure the interfacial concentrations of water and halide counterions in aqueous cationic micelles and microemulsions. However, Romsted's method for cationic surfactant assemblies can not measure the local molar concentrations of the surfactant head-groups. In contrast, the present method can measure the local molar concentrations of sulfosuccinate head groups and water in anionic AOT–2,2,4-trimethylpentane–water reverse micelles, but can not measure the interfacial concentrations of sodium counterions.

Conclusion

In summary, in the present work using dediazoniation reactions as the trapping reaction, we have obtained the first "snap-shot" of the local molar concentrations of water and sulfosuccinate head-groups in the interfacial region of anionic AOT reverse micelles. In the process, we have determined the structural identity of the new dediazoniation products formed in the AOT–2,2,4-trimethylpentane–water reverse-micelle associated dediazoniation of 16-ArN_2^+ by its actual isolation and have prepared the hitherto unavailable AOT head-group mimic, the water-soluble sodium sulfosuccinate salt. Our results demonstrate that the interfacial regions of anionic AOT–2,2,4-trimethylpentane–water reverse micelles are densely populated by the sulfosuccinate head-groups of AOT and the interfacial concentrations of water in AOT reverse micelles are significantly lower than the molar concentration of bulk water. Thus, our results throw new light on the microenvironments of interfacially located enzymes such as lipases entrapped in AOT–2,2,4-trimethylpentane–water reverse micelles, the most extensively exploited reverse-micellar system in micellar biotechnology.

Experimental Section

Materials: 16-ArN_2BF_4, 1-ArN_2BF_4, and 16-ArOH were synthesized as described previously.[23] 1-ArOH was purchased from Aldrich and was further purified by recrystallization from ethyl acetate and petroleum ether. All of the other reagents used in the synthesis were procured from Spectrochem (India) and were of the highest analytical grade. All of the solvents used were of HPLC grade and were purchased from Qualigens (India).

General methods: ^{1}H NMR spectra were recorded on a Varian FT 200 MHz spectrometer and the high-resolution mass spectrometric (HRMS) analyses were performed on a Micromass AUTO SPEC-M mass spectrometer (Manchester, UK) with OPUS V3.1X data system.

Isolation and characterization of 1-ArS: The product 1-ArS was isolated by carrying out the dediazoniation reaction of 1-ArN_2^+ (0.1 M) in aqueous SDSS (25 mL, 1.0 M) for 24 h at room temperature in the dark. The product mixture was extracted with diethyl ether (3 × 50 mL), the combined ether extracts were washed with water (3 × 100 mL) and dried with anhydrous sodium sulfate. Diethyl ether was removed from the dried ether extract by rotatory evaporation and the residue was dissolved in HPLC-grade acetonitrile (1 mL). The product 1-ArS was finally purified by semipreparative HPLC. The acetonitrile solution of the product mixture (1 mL) was injected into a Whatman Partisil 10 ODS-3 column using a 1 mL sample loop, 60:40 (v/v) acetonitrile/water as the mobile phase at a flow rate of 8 mL min^{-1} and at a UV-detector wavelength of 230 nm. The peak was measured after 36.0 min. Evaporation of the mobile phase using rotatory evaporation followed by vacuum drying of the residue afforded pure 1-ArS (160 mg, white solid). 1-ArS: ^{1}H NMR (200 MHz, CDCl$_3$): δ = 2.25 (s, 3H; p-CH_3), 2.35 (s, 6H; o-CH_3), 3.15–3.55 (2dd, 2H; CHCH_2CO), 3.8 (s, 3H; CH$_2$COOCH_3), 3.9 (s, 3H; OSO$_2$CH(COOCH_3)CH$_2$), 4.6–4.7 (dd, 1H; OSO$_2$CH(COOCH$_3$)CH$_2$), 6.85 (s, 2H; ArH); HRMS (LSIMS) m/z: calcd (for C$_{15}$H$_{21}$O$_7$S) 345.1041, found 345.1008.

Isolation and characterization of 16-ArS: The product 16-ArS was isolated by carrying out the dediazoniation reaction of 16-ArN$_2^+$ (4.5 mM) in AOT (100 mL, 0.1 M)–2,2,4-trimethylpentane–water (W_0 = 20) reverse micelle for 24 h at room temperature in the dark at pH 7 (25 mM, Tris/HCl). 2,2,4-Trimethylpentane was evaporated and the dediazoniation products were extracted from the residue with acetonitrile (50 mL), then centrifuged and the supernatant was removed. The precipitate (mostly AOT) was further extracted with acetonitrile (2 × 50 mL), and acetonitrile was removed from the combined acetonitrile extracts by rotatory evaporation. The remaining residue was dissolved in HPLC-grade methanol (1 mL), and the product 16-ArS was finally purified by semipreparative HPLC. The methanol solution of the product mixture (1 mL) was injected into a Whatman Partisil 10 ODS-3 column using a 1 mL sample loop, 80:20 (v/v) methanol/propan-2-ol as the mobile phase at a flow rate of 8 mL min^{-1} and at a UV-detector wavelength of 219 nm. The peak was measured after 28.7 min. Evaporation of the mobile phase using a rotatory evaporator followed by vacuum drying of the residue afforded pure 16-ArS (50 mg, white solid). 16-ArS: ^{1}H NMR (200 MHz, CDCl$_3$): δ = 0.9 (t, 15H; CH$_3$), 1.2–1.5 (m, 42H; (CH_2)$_n$), 1.5–1.8 (m, 4H; OCH$_2$CH(CH$_2$CH$_3$), p-CH$_2$CH_2(CH$_2$)$_{13}$CH$_3$), 2.35 (s, 6H; o-CH_3), 2.5 (t, 2H; p-CH_2CH$_2$), 3.2–3.6 (2dd, 2H; OOCCH_2CH(SO$_3^-$)), 4.05 (d, 2H; CH$_2$COOCH_2CH(CH$_2$CH$_3$), 4.2 (d, 2H; (ArOSO$_2$)CHCOOCH_2CH(CH$_2$CH$_3$)), 4.6–4.75 (dd, 1H; OOCCH$_2$CH(SO$_3^-$)), 6.85 (s, 2H; ArH); HRMS (LSIMS) m/z: calcd (for C$_{44}$H$_{79}$O$_7$S) 751.5546, found 751.5486.

Synthesis of sodium dimethylsulfosuccinate (SDSS): Maleic anhydride (10 g, 0.1 mol) was treated with excess methanol (100 mL) plus concentrated sulfuric acid (10 drops) under refluxing conditions overnight. The solvent was removed by rotatory evaporation, and the residue was dissolved in ethyl acetate (50 mL). The ethyl acetate solution was washed first with saturated aqueous NaHCO$_3$ (100 mL) and then with water (3 × 100 mL), dried over anhydrous sodium sulfate, filtered, and ethyl acetate was removed from the filtrate by using a rotorary evaporator. The residue (dimethyl maleate, 11 g, 0.08 mol) was dissolved in methanol/water (100 mL, 90:10 (v/v)), and sodium bisulfite (9.7 g, 0.09 mol) was added to the resulting solution. The mixture was then kept under refluxing conditions overnight. The solvent was rotavapored, the residue was stirred with methanol (100 mL) and filtered. The precipitate (mostly unreacted NaHSO$_3$) was extracted further with methanol (2 × 100 mL) and methanol was removed from the combined methanol extracts by using a rotary evaporator. The residue was kept under vacuum pump for an hour. The title compound was finally obtained as pure white crystals (6.4 g, in 25% overall yield) after three consecutive recrystallizations of the residue from methanol/ethyl acetate. SDSS: ^{1}H NMR (200 MHz, D$_2$O): δ = 2.8–2.9 (m, 2H; ($^-$OSO$_2$)CHCH_2COO), 3.4 (s, 3H; CHCH$_2$COOCH_3), 3.5 (s, 3H; ($^-$OSO$_2$)CHCOOCH_3), 3.9–4.1 (dd, 1H; ($^-$OSO$_2$)CHCH$_2$COO); HRMS (LSIMS) m/z: calcd (for C$_6$H$_9$O$_7$SNa$_2$) 270.9864, found 270.9879

HPLC: Product distribution analyses were carried out with a Shimadzu HPLC system, model LC10A using 20 μL sample loop. The wavelength of the UV detector was set at 230 nm for the detection of products from the dediazoniation of 1-ArN$_2^+$ and 219 nm for the detection of products from the dediazoniation of 16-ArN$_2^+$. The separation of the products obtained from the reverse-micelle associated dediazoniation of 16-ArN$_2^+$ were effected on a millipore water steel cartridge (Nova Pak) Partisil 5 ODS-3 WCS analytical column (4.6 × 250 mm). Methanol/propan-2-ol (80:20, v/v) was used as the mobile phase with a flow rate of 0.8 mL min^{-1}. Typical column pressure was 54–56 bar; typical retention times were: 16-ArOH: 6.3 min; 16-ArS: 9.9 min. The mobile phase used for the determination of the product distributions in nonmicellar aqueous dediazoniation of 1-ArN$_2^+$ was acetonitrile/water (70:30, v/v) with a flow rate of 0.6 mL min^{-1}. Typical column pressure was 52–54 bar; typical retention times were: 1-ArOH: 7.7 min; 1-ArS: 9.5 min. The identities of the peaks in the HPLC chromatograms were confirmed by spiking experiments with pure 16-ArOH and 16-ArS in the case of reverse-micellar dediazoniation of 16-ArN$_2^+$, and with pure 1-ArOH and 1-ArS in the case of nonmicellar dediazoniation of 1-ArN$_2^+$ in the presence of SDSS.

Reverse-micelles associated dediazoniation reactions of 16-ArN$_2^+$: The reverse-micellar solution was prepared in a 10 mL volumetric flask by addition of AOT (444.5 mg, 1 mmol) to the required volume of aqueous Tris buffer (25 mM, pH 7.0) as the water pool to maintain the particular W_0. The volumetric flask was filled up to the mark with 2,2,4-trimethylpentane followed by vortexing for 10–15 s. Interfacially associated thermal dediazoniation of 16-ArN$_2^+$ was initiated by the addition of 16-ArN$_2^+$ (ca. 4.4 mg) to reverse micelles (10 mL) to give a reaction concentration of 16-ArN$_2^+$ of 1 × 10^{-3} M. All the dediazoniation reactions were carried out for at least 10 half lifes (5 days at room temperature), and the dediazoniation product mixtures were diluted ten times with methanol before injecting into HPLC.

Nonmicellar aqueous dediazoniation reactions of 1-ArN$_2^+$: The nonmicellar aqueous SDSS solutions (0.2–0.85 M) were prepared in 2 mL volumetric flasks by taking the required volumes of the stock solution of SDSS in water (1.02 M) and filling the volumetric flask up to the mark with water. The thermal nonmicellar dediazoniation was initiated by addition of the stock solution of 1-ArN$_2$ $^+$ (20 μL, 0.1 M in acetonitrile) into the aqueous solution (2 mL) so that the reaction concentration of 1-ArN$_2^+$ was 1 × 10^{-3} M. All the dediazoniation reactions were carried out for at least 10 half lifes (5 days at room temperature) and the dediazoniation products were diluted ten times with the mobile phase before injecting into HPLC.

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