J. Indian Chem. Soc.,
Vol. LVIII, July 1981, pp. 633-639

Water inside Reverse Micelles†

D. BALASUBRAMANIAN*

Centre for Cellular and Molecular Biology, Regional Research Laboratory, Hyderabad-500 009

(a) *MICELLES :* The aggregation of amphiphilic substances in aqueous solution is a well-known phenomenon that has been studied both for its intrinsic structural interest as well as for its relevance and applications in detergency, solubilization, interfacial thermodynamics and kinetics, catalytic properties, biological modelling, compartmentalization of substances, and phase behaviour. An amphiphile is a compound that possesses a hydrophobic nonpolar moiety (tail) and an ionic or dipolar hydrophilic head-group. As a result of this dual structural feature, amphiphilic compounds are surface-active and are referred to as surfactants. Surfactant molecules are classified according to their charge type as anionic (e.g., sodium dodecyl sulphate, SDS), cationic (e.g., cetyltrimethylammonium bromide, CTAB), nonionic (e.g., polyethylene glycol ethers such as Brij or Triton that bear a hydroxyl end-group), or zwitterionic, such as lecithin—the phospholipid that is the predominant lipid component of biological membranes and vesicles. Surfactant aggregation in water solution is a phenomenon driven by the hydrophobic effect[1] resulting in micelles wherein the hydrophilic groups are exposed to the aqueous phase and the core offers itself as a nonpolar hydrocarbon environment that can solubilize significant amounts of 'oil' in its interior. Since hydrophobic association is nonspecific, a variety of nonpolar substances can be solubilized in aqueous media using surfactant micelles. The micellar phase can thus be seen to be of considerable chemical interest and utility.

(b) *Reverse micelles :* The related phenomenon of reverse micelles, i.e., the aggregation of surfactant molecules in nonpolar organic solvents, is one that elicits surprise. While reverse micelles have been noticed and studied in some detail since over thirty years ago[2], the driving force for such aggregation is not well understood. Reverse micellar aggregation is not as common as regular micelles, and involves special features of the surfactant molecule, e.g., hydrophile-lipophile balance (HLB)[3], the bulk solvent, temperature, and other conditions, but it is apparent that hydrophobic association cannot be responsible for aggregation in these nonpolar structureless solvent media. There are suggestions that the presence of water may be essential for reverse micelles to form[4], and that there may be no definite critical reverse-micellar concentration required in these cases[5]. These points are particularly relevant to the issue of (i) why all surfactants that form micelles in water do not form reverse micelles in organic media, (ii) the kind of HLB needed for reverse-micellization, (iii) the role and status of water, both in trace amounts and in significantly solubilized amounts, in reverse micelles and (iv) whether the structural features of the surfactant molecule and its segments in a reverse micelle are comparable to those in its regular micelles in water.

(c) *The water pool :* A point of particular interest is the status of the water that is solubilized in reverse micelles. Several surfactants, e.g., Aerosol OT (Na diethylhexyl sulphosuccinate)[6], dodecylammonium propionate[7], polyoxyethylene alkylethers[8], CTAB[9] and lecithin[10] aggregate to produce reverse micelles in nonpolar media and are able to solubilize significant amounts of water into them, often as high as 50 molecules of water per molecule of surfactant. The water is thought to form a 'pool' in the core of the reverse micelle, interacting with the polar/ionic head groups of the surfactant and insulated from the bulk solvent by a monomolecular surfactant sheath. The properties of this compartmentalized water are of interest, since the amount of solubilized water can be varied at will; a fraction of it would be bound to the surfactant while the rest would be 'free' or 'bulk-like'. The water pool is formally akin to the water found in natural membranous pockets, the cytoplasmic aqueous phase, the mitochondrial matrix, lysosomal inner phase and the inner aqueous phase of liposomes.

(d) *Why nonionic surfactants :* A disadvantage in using ionic surfactants in reverse micellar studies is that the solubilized water would be expected to be strongly bound to the charged head groups through ionic hydration and electrostriction, thereby making it already substantially different from bulk water. In order to study the nature of the pool water, it would thus be desirable to avoid effects due to ionic interactions. Further, when one wishes to study the properties of biopolymers, such as enzymes, dissolved in the water pool, absence of charge interactions would make interpretations easier and less ambiguous. We have hence chosen to use reverse micelles of nonionic surfactants to study the water pool.

(e) *Microemulsions :* A difficulty that one faces when trying to study reverse micelles of several nonionic detergents is that these do not

† The Yeddanapalli Memorial Award Oration, delivered at the Convention of Chemists, India, held at the I. I. T., Powai, Bombay, on December 10, 1980.

* On leave from the School of Chemistry, University of Hyderabad, Hyderabad-500 134.

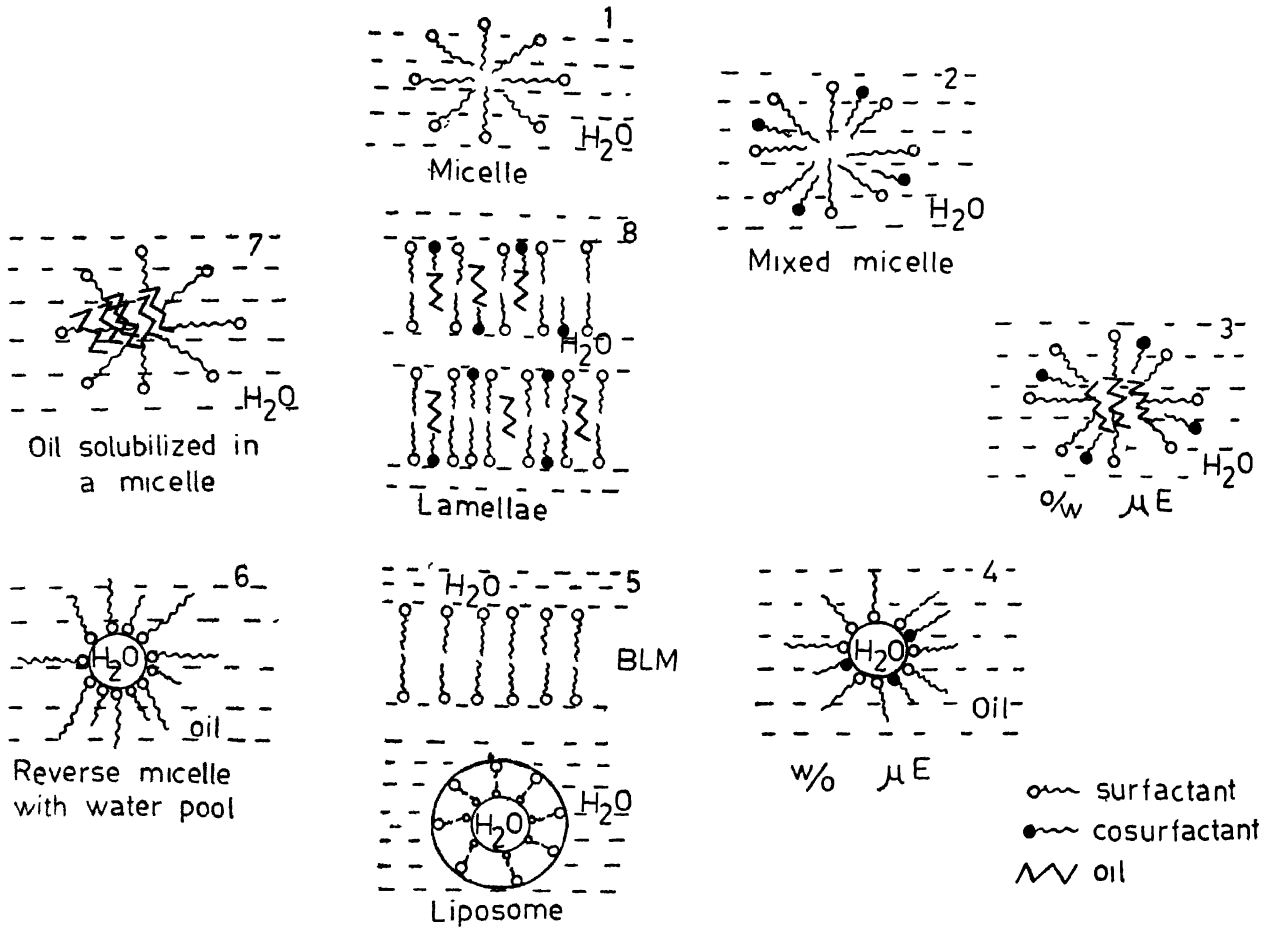

Fig. 1. Pictorial representation of the various modes of aggregation of surfactant molecules. 1, 2, 3, 5 and 7 are dispersed in bulk aqueous phase. 4 and 6 are in oil as the bulk phase. 8 has about equal amount of oil and water. The mixed reverse micelle (inverse of 2) is not shown.

generally aggregate to beyond oligomeric levels in nonpolar media. This difficulty can be overcome by the addition of a proper cosurfactant compound which aids in optimizing the HLB and produces 'mixed micelles' or 'mixed reverse micelles'. Traditionally such four-component systems (surfactant, cosurfactant, water and 'oil', i.e., nonpolar solvent) are referred to as microemulsions[11]. When the bulk phase is water and the surfactant : cosurfactant mixture aggregates to produce micellar entities solubilizing oil within, such systems are called oil-in-water (o/w) microemulsions, and while the reverse occurs wherein water is solubilized through surfactant : cosurfactant aggregation in a bulk oil phase, they are termed water-in-oil (w/o) microemulsions. Shah and coworkers[12-15] have shown by a comparative study that the structural features of a o/w microemulsion are very similar to those of a regular micelle in aqueous medium, and we have shown in a series of recent papers[8, 16-18] that there is little structural difference between a w/o microemulsion and a reverse micelle. In effect, as Friberg has shown by phase diagrams[19], microemulsions are simply swollen micellar assemblies, albeit with a higher solubilization capacity[15]. Our detailed study and comparison has shown that both in structural dynamics and organizational characteristics, w/o microemulsions are very similar to reverse micelles. In light of these, and the fact that nonionic surfactants can be easily coaxed to form w/o microemulsions, we focus attention here on the properties of the water pool encapsulated in the w/o microemulsions or swollen reverse micelles of the system Triton X-100 : hexanol in cyclohexane.

(f) *Advantages of the system for biological modelling :* There are several attractive features to a reverse micellar (or w/o microemulsion) water pool, when one wishes to study it as a model for biologically encapsulated water. The traditional model for the purpose has been the liposome[20,21], which is a dispersion in aqueous medium of a spherical or non-spherical bimolecular leaflet of a surfactant lipid with an inner compartmentalized aqueous phase (see Fig. 1 for a pictorial representation of the several modes of aggregation of surfactants). A typical stable liposome has a diameter ranging from 500 to 2000 Å, thus making it scatter

light of wavelengths of optical spectroscopic interest (wavelengths 2000-10000 Å). Also the leakage rate of several types of molecules encapsulated within the inner aqueous phase of liposomes is very low, thereby making them essentially insulated from the external medium. In contrast, reverse micelles and w/o microemulsions are far smaller in size (50-200 Å diameter) which makes them optically non-scattering and transparent. Reverse micelles are in effect half-liposomes, and the molecules trapped in their water pools *via* the monomolecular surfactant sheath are accessible by ordinary diffusion and coalescence mechanisms. These are two distinct advantages that at once make the water pool and its contents accessible to studies by optical spectroscopic methods. We have exploited these advantages and present the results of such studies in subsequent sections.

We emphasize here studies on water pools of the swollen reverse micellar system prepared from the nonionic surfactant Triton X-100, since this is the first of its kind on a nonionic system where the pool water would be expected not to be perturbed in its nature by ionic effects or electrostriction. We have also shown earlier[17,18] that w/o microemulsions are essentially not different in their structural or organizational properties from reverse micelles.

Experimental

The method of preparation of w/o microemulsions of Triton X-100 (with hexanol as cosurfactant) and their characterization have been detailed in earlier papers[8,16] from this laboratory. Briefly, to a 20% solution (w/v) of Triton X-100 : hexanol (4 : 1 v/w ratio) in dry cyclohexane are added increasing amounts of water, shaken in a vortex mixer, and studied. For this surfactant composition, the microemulsions are isotropic clear spherical aggregates with the size increasing with increasing water solubilized. At 10% water, the system displays a phase transition from spherical reverse micelles to a lamellar anisotropic multibilayer liquid crystalline phase that is stable until 18% added water (v/v), beyond which its phase separates into a milky macroscopic emulsion[8]. Thus our studies are confined to water contents below 18% by volume.

The near infrared (NIR) and UV-visible spectral studies were made using a Cary 17-D spectrophotometer, the fluorescence measurements using both a home-built instrument and a Hitachi spectrofluorimeter, NMR studies using a JEOL FX-100 FT instrument using the 180-τ-90 pulse sequence for relaxation time measurements using the dedicated computer, and circular dichroism spectra using a JASCO J-20 spectropolarimeter.

Results and Discussion

(i) *Status of the water :* A major advantage of using nonionic surfactants for making reverse micelles is that, in these systems, one can avoid ionic hydration and electrostriction of water, thereby making a study of the water pool more convenient. One would like to estimate the fraction of water molecules that are bound to the surfactant molecules (head groups, and in the case of polyoxyethylene glycol surfactants such as Triton X-100, the oxyethylene segments as well) and the fraction that might behave as 'free' or like bulk water. To this end, we have studied the nuclear magnetic resonance absorption and relaxation (spin-lattice or longitudinal relaxation, T_1) processes of the water protons and of the ethylene oxide protons of Triton X-100, and have summarized the results in Table 1. In the absence of any added water, the ethylene oxide protons show a sharp signal at 3-84 δ agreeing with those of Triton X-100 in cyclohexane, and presumably indicative of a true molecular (unaggregated) solution of the surfactant in the medium. Addition of as little as 2% water (water : Triton X-100 molar ratio of 4.3) causes a downfield shift of the signal to 3.92 δ, and further addition of water causes a gradual downfield shift to a limiting value of 4.00 δ. From this change, it appears that as the microemulsion forms and swells in size upon water addition, the ethylene oxide groups are moved from a nonpolar to a more polar environment, presumably an aqueous one. Similar results have been

TABLE 1—NMR ANALYSIS OF THE TRITON X-100 REVERSE MICELLAR SYSTEM IN CYCLOHEXANE

Water added v/v	$R=\frac{[H_2O]}{[-TX]}$	δ_{OH} ppm	$\delta_{CH_2CH_2O}$ ppm	T_1(OH) s	T_1(CH_2CH_2O), s	$\tau_c(H_2O)$ $\times 10^{10}$ s	η of pool, in CP	$\frac{[H_2O]\ \text{bound}}{[\text{EO unit}]}$
0	0	—	3.84	(0.4)	1.10	—	—	—
0.5	1.04	4.10	3.85	—	0.65	—	—	—
1.0	2.09	4.40	3.90	0.45	0.45	0.39	9.7(5.5)	0.16
2.0	4.17	4.60	3.92	0.50	0.38	—	—	0.24
3.0	6.26	4.65	3.93	0.60	0.34	0.30	7.7(4.4)	0.33
4.0	8.34	4.80	3.94	0.65	—	—	—	0.40
5.0	10.43	—	3.95	0.70	0.34	0.26	6.6(3.8)	0.47
6.0	12.52	4.85	3.95	0.82	—	—	—	0.50
7.0	14.60	—	3.96	0.90	0.36	0.19	4.8(2.7)	0.52
8.0	16.69	4.92	3.96	0.95	—	—	—	—
9.0	18.8	4.95	3.96	1.00	0.34	0.18	4.4(2.5)	—
10.0	20.9	5.00	3.98	(0.90)	(0.34)	—	—	—

obtained with Tween 80 (another polyoxyethylene based surfactant) reverse micelles in xylene[22]. Turning next to the OH protons, the fact that one observes a single signal indicates that there is fast exchange of protons between water, Triton X-100 terminal hydroxyl groups and hexanol. But since the concentration of water protons is about ten-fold higher than that of the others even at 2% added water, we are interpreting the OH signals as those due to water itself.

As can be seen from Table 1, the OH proton signal also exhibits a downfield shift from 4.1 δ at 0.5% water to 5.0 δ, the bulk water value, at 8% added water and beyond. This downfield shift suggests that as the microemulsion forms and the water pool increases in size, the chemical environment of the water molecules changes and a large fraction of the pool water becomes similar to bulk liquid water. But, as we shall see, the mobility and the average polarity of the pool water appear lower than those of neat liquid water. Finally, the presence of a single OH signal suggests that the residence time of water in the pool and in interfacial sites is shorter than 10^{-4} s.

(ii) *Microstructural mobility by T_1 measurements :* An indication of the mobility of the segments of a molecule is offered by following the spin-lattice relaxation time (T_1) of the constituent NMR nuclei. A large value of T_1 indicates high mobility, while small values of T_1 suggest restricted motion or 'freezing'. Table 1 reveals that the T_1 values of the surfactant backbone oxyethylene protons drop from an initial value of 1.1 s to about 0.3 s by the time 2% water is added, and show no significant variation beyond this. This would mean that the surfactant suffers a loss of mobility (from the true solution type in dry cyclohexane) upon aggregation to form the reverse micelle, and this microstructural restriction in its mobility is not vastly changed in nature as the swelling of the reverse micelle occurs upon increasing water addition, or even when the system changes its phase to produce lamellae (beyond 10% water). The relaxation times of the water protons, on the other hand, increase from 0.45 s at 1% water to 1.0 s at 9% water, suggesting an initial immobilization of the water that becomes increasingly free as the pool size increases. The pool water, judging from its T_1 values, is not as mobile as bulk water is (whose T_1 in the neat liquid phase is over 3s).

An analysis of the OH proton T_1 values by the theory of dipolar magnetic relaxation gives an idea about the ease of molecular motion and the microviscosity within the pool. T_1 is related to the reorientational correlation time, τ_c as[23] :

$$\frac{1}{T_{1\,\text{intra}}} = \frac{3}{10}\frac{\gamma^4 \hbar^2}{b^6}\left[\frac{\tau_c}{1+\omega^2\tau_c^2} + \frac{4\tau_c}{1+4\omega^2\tau_c^2}\right] \quad \ldots (1)$$

$$\frac{1}{T_{1\,\text{inter}}} = 9\pi^2\gamma^4\eta\hbar^2\,\text{No}/5\text{kT} \quad \ldots (2)$$

$$\frac{1}{T_1} = \frac{1}{T_{1\,\text{intra}}} + \frac{1}{T_{1\,\text{inter}}} \quad \ldots (3)$$

where $T_{1\,\text{inter}}$ are the intra- and intermolecular dipolar relaxation times, γ the magnetogyric ratio, b the interproton distance in the water molecule, τ_c the reorientational correlation time, ω the resonance frequency, η the viscosity of the medium surrounding the spin, N_o the concentration of the spins per cm^3, k the Boltzman constant and T the temperature. If one assumes water to hydrodynamically move as a sphere of radius a, then τ_c and the microviscosity η are related as

$$\tau_c = 4\pi\eta\, a^3/3kT \quad \ldots (4)$$

we have analysed the T_1 of water protons, using the above formalism and calculated τ_c and η in the water pools, which are given in Table 1. η has been calculated using equation (1) alone and also by including intermolecular modes (equations 2 and 3), the latter values being given in parantheses. It is noteworthy that τ_c of water is in the range $0.2\text{-}0.4 \times 10^{-10}$ s, suggesting that the motion of the water is independent of that of the reverse micelle, which is expected to have values in the range of 10^{-9} s or greater. Also, as the pool size increases the microviscosity decreases, suggesting greater fluidity in the pool.

(iii) *Hydration of the surfactant :* The fact that the ethylene oxide proton signals show a downfield shift with increasing addition of water would suggest interaction between the ether oxygens and water molecules, probably by hydrogen bonding, and would be expected to level off beyond a certain amount of added water. Since the water molecules that are bound would experience a mobility that is different from those that exist as 'bulk' water, it should be possible to analyse the T_1 values of water protons in terms of a two-site model and estimate the fraction of water molecules bound to the surfactant. We have done such an analysis and have expressed the bound water fraction in units of moles of water bound per mole of the surfactant in Table 1. Compared to this figure of 5-5.5 molecules of water bound per Triton X-100 in reverse micellar form is the hydration number of 14 that is estimated in aqueous micelles of the same detergent at room temperature[24].

(iv) *Polarity of the water pool :* The polarity of the water solubilized within reverse micelles has been probed in the past using absorption or emission probe molecules which are large and whose location is subject to doubt. We have therefore used the NO_3^- anion as a small and specifically water-soluble probe whose polarity-dependent $n \rightarrow \pi^*$ absorption band maximum can be utilized to monitor the polarity and hydrogen-bonding ability of the pool water. The variation in the absorption maximum of solubilized KNO_3 with the amount of added water in the present system was compared with the solvent dependent band maximum of quaternary alkyl-ammonium nitrates[25], and the polarity of the water pool estimated in terms of the Kosower[26] solvent parameter Z. Such a plot of the Z values of the water pool is shown in Fig. 2. It can be seen that when the water content is 1%

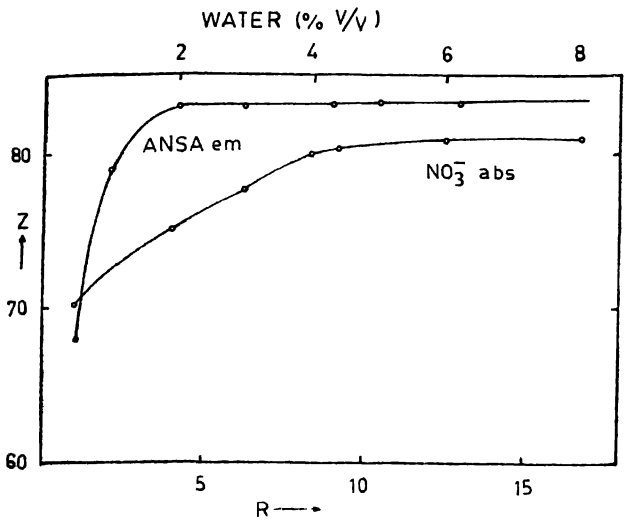

Fig. 2. Variation in the solvent polarity index, Z, of the pool water as estimated by the fluorescence maximum of ANSA and by the absorption maximum of KNO_3. The system is Triton X-100 : hexanol 4 : 1) in cyclohexane.

(water : Triton X-100 molar ratio = R = 2.2), the polarity of the pool is quite low, the Z value being about 71 and rapidly increases to the limiting Z value of about 82-83. Even at the highest pool size tried (R = 17), the polarity does not approach the Z value of 94.6 seen in pure liquid water. The water pool appears to be less polar than bulk water.

Solvent-dependent shifts are larger in magnitude in fluorescence spectra (both in the emission maxima and in quantum yields) than in absorption, and a common fluorescence probe chosen in micellar systems is 1-anilino-naphthalene-8 sulfonate (ANSA), whose emission characteristics have been studied as a function of Z values of several solvents[27]. When ANSA was incorporated in the present microemulsions, it showed an emission maximum at 458 nm (quantum yield $\phi = 0.39$) when no water at all was present, corresponding to a Z value of about 65. Addition of 2% water (R = 4.4) causes the band to redshift to 488 nm ($\phi = 0.25$) corresponding to a Z value of around 80. The largest pool tried (R = 15.2, 7% water) seemed to have a polarity of Z = 84, roughly that of methanol. The variation of the polarity of the water pool as measured by ANSA fluorescence is shown in Fig. 2. It is interesting to note that the shape of the polarity variation curve as monitored by ANSA emission differs in its profile from that measured by the nitrate absorption, though the final levelling-off regions appear compatible. This difference might owe its origin to the possibility that while KNO_3 resides entirely in the water pool, the fluorescent probe might be surface active and insert itself in the interface, in proximity to the oxyethylene segments of Triton X-100.

(v) *The pH of the pool :* In light of the reduced polarity and mobility displayed by the pool water, it is of interest to inquire into its acidity, or its internal *p*H. Here again, it is of advantage to use a nonionic surfactant to form the water pool than an ionic one, since in the latter complications might arise due to the local amplification of the ion concentration and the sequestering of a part of the pool water by ionic hydration—factors that might alter the action of buffer solutions that are encapsulated within. It has been shown that at low water contents, the internal *p*H of the water pools of the anionic detergent Aerosol OT in heptane is larger than that of the original buffer solution used for solubilization, and that the two *p*H values become equal only when the pool size is quite large[28]. Fendler has found[29], using fluorescence analysis, that the internal *p*H of the inner aqueous phase liposomes (*p*Hi) to be the same as that of bulk (*p*Ho), and *p*Hi > *p*Ho for cationic liposomes while *p*Hi < *p*Ho for anionic liposomes. We have used three indicator dyes as *p*H probes to check the internal *p*H of the water pool of Triton X-100 reverse micelles[16]. The absorption spectra of the dyes solubilized as aqueous solutions at different *p*H values, within the water pools of the present system showed no difference (to within 0.2 *p*H units) from their bulk water values. These results suggest that though a significant fraction of the pool water is bound to the Triton head group, the internal acidity is not significantly different from that of bulk water. The pool water of non-ionic reverse micelles is thus likely to be the same as bulk water in its acidity.

(vi) *The water pool as solvent for proteins :* One of the advantages of working with reverse micelles is that these are optically transparent and thus offer a study of the proteins encapsulated in the water pool easier in comparison to those in liposomes that scatter light. It has thus been possible, for the first time, to investigate the trapped protein by absorption spectroscopic means and by enzyme assay methods using reverse micelles – a study that has not been possible using liposomes. We choose to briefly illustrate here such an investigation on the globular protein molecule α-chymotrypsin, a proteolytic enzyme.

Solubilization of the enzyme in the pools was achieved by adding a measured amount of protein dissolved in a buffer (*p*H 7.4, 100 mM phosphate) to a measured volume of the surfactant in a non-polar solvent and mixing gently to ensure homogeneity. The conformation of the enzyme in the pool was monitored by circular dichroism (CD) spectroscopy, and its enzymatic activity was determined by following the release of the phenol formed by the catalytic hydrolysis of 2, 4 dinitrophenyl acetate spectrophotometrically, appropriately corrected for spontaneous hydrolysis and using suitable blanks, using the Bender-Kezdy procedure[30].

Fig. 3 shows the fraction of the activity of the enzyme retained (with reference to the aqueous buffer control) in the water pools of the Triton X-100 system, as the size of the pool is increased to reach the lamellar phase. The enzyme is seen to

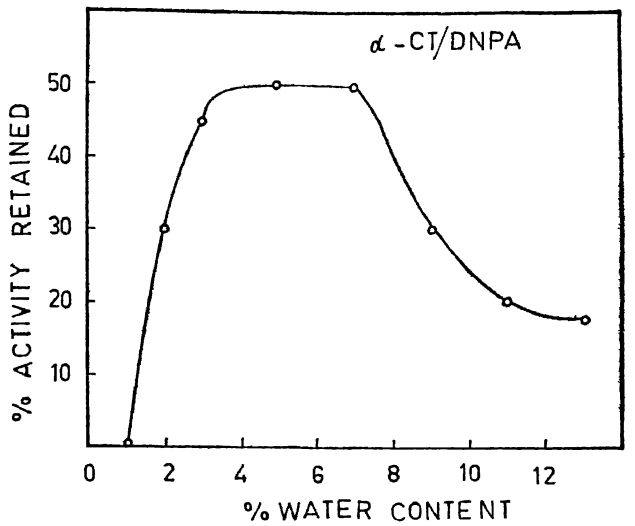

Fig. 3. Enzyme activity profile in the water pool, as compared to aqueous buffer activity, of chymotrypsin. The substrate is 2, 4-dinitrophenyl acetate. The system in Triton X-100 : hexanol (4 : 1) in cyclohexane.

maintain significant amounts of its activity even in the lamellar phase, a fact that explains the interesting photoregulation behaviour exhibited by the enzyme in a model membrane lamella of oleate in the presence of azobenzene, observed by us earlier[31].

TABLE 2—ENZYMATIC ACTIVITY AND CONFORMATIONAL STATUS OF α-CHYMOTRYPSIN IN WATER POOLS

Reverse micellar system	Water concentration v/v	Enzyme activity[1] w.r t. water	Conformation in pool[2], as per C.D. spectra
Aerosol OT (5% in octane)	1%	0%	–
	3%	32%	–
	5%	51%	partly retained
Na Laurate (5%+10% hexanol, in cyclohexane)	2% and above	0%	modified
CTAB (5%+10% hexanol, in cyclohexane)	2% and above	0%	modified
SDS (5%+10% hexanol, in cyclohexane)	2%	59%	Retained
	3%	85%	Retained
	5%	85%	Retained
Brij 56 (15%+6% hexanol in cyclohexane)	0.5%	65%	–
	1%	87%	–
	3%	86%	Retained

1 Substrate : 2,4 dinitrophenyl acetate ; *p*H 7.4 phosphate buffer ; comparison with aqueous buffer control ; accuracy ±10%

2 'modified' : all features of the water CD spectrum lost and low ellipticities seen.
'retained' : Spectrum in the pool resembles in all features the CD spectrum in aqueous buffer.
'partly retained' : CD of the protein in the pool is similar in features, but lower in magnitude, to that in aqueous buffer solution. All comparisons are with aqueous buffer solution of the protein.

This kind of experiments also explain the earlier reports of enzymes exhibiting activity in nonpolar solvents in the presence of certain surfactants[32]. In Table 2, we show the extent of activity and also the conformational status of chymotrypsin in a variety of reverse micellar systems, anionic, cationic and uncharged. The variations seen in the activities, and the CD spectral features, in the various water pools seem to raise from several factors—such as changes in the internal *p*H of the pool, possible protein-surfactant head group interactions and the like. We are currently investigating this problem in some detail. The fact that pools of the two non-ionic systems—Triton X-100 and Brij 56-retain the enzyme conformation and function well suggests that a soft head group and the interfacial barrier by the polyoxyethylene segments might be conductive for the enzyme to keep its structural and hence functional integrity. The utility of enzymes encapsulated within water pools would appear diverse, and is being currently explored in our laboratory and will be reported elsewhere.

Acknowledgement

Most of the work reported have been carried out by Dr. C. Kumar. It has been a pleasure developing the ideas and the implications of the work with him. Mr. P. Mitra is continuing on with some of this and related work. I am thankful to him for criticism of the manuscript. This work has been aided by a grant from the Science and Engineering Research Council of the Department of Science and Technology, to whom we express our gratitude.

References

1. C. TANFORD, 'The Hydrophobic Effect', Wiley-Interscience, New York, 1973.
2. S. R. PALIT, *J. Colloid Sci.*, 1949, **4**, 523 ; S. R. PALIT and V. VENKATESWARLU, *Proc. Roy. Soc.*, 1951, **A208**, 542 ; S. R. PALIT and V. VENKATESWARLU, *J. Chem. Soc.*, 1954, 2129.
3. K. KON-NO and A. KITAHARA, *J. Colloid Interface Sci.*, 1972, **41**, 47, 86.
4. H. F. EICKE and H. CHRISTEN, *Helv. Chim. Acta*, 1978, **61**, 2258.
5. N. MULLER, *J. Phys. Chem.*, 1975, **79**, 287.
6. M. WONG, M. GRATZEL and J. K. THOMAS, *J. Amer. Chem. Soc.*, 1976, **98**, 2391.
7. J. H. FENDLER, *Acc. Chem. Res.*, 1976, **9**, 153.
8. C. KUMAR and D. BALASUBRAMANIAN, *J. Colloid Interface Sci.*, 1979, **69**, 271.
9. J. SUNAMOTO, *Bull. Chem. Soc.*, Japan, 1978, **51**, 3130.
10. M. A. WELLS, *Biochemistry*, 1974, **13**, 4937.
11. L. M. PRINCE, 'Microemulsions—Theory and Practice', Academic Press, New York, 1977.
12. D. O. SHAH, *Ann. N. Y. Acad. Sci.*, 1973, **204**, 125.
13. J. W. FALCO, R. D. WALKER, JR., and D. O. SHAH, *A. I. Ch. E. J.*, 1974, **20**, 510.
14. D. O. SHAH, R. D. WALKER, JR., W. C. HSIEH, N. J. SHAH, S. DWIVEDI, J. NELANDER, R. PEPINSKY and D. W. DEAMER, SPE-5815 at 'Improved Oil Recovery Symposium', Tulsa OK, USA, 1976.
15. D. O. SHAH, V. K. BANSAL, K. CHEN and W. C. HSIEH, in 'Improved Oil Recovery by Surfactant and Polymer Flooding', Eds. D. O. SHAH and R. S. SCHECHTER, Academic Press, New York, 1977, p. 293.
16. C. KUMAR and D. BALASUBRAMANIAN, *J. Colloid Interface Sci.*, 1980, **74**, 64.

17. C. KUMAR and D. BALASUBRAMANIAN, *J. Phys. Chem.*, 1980, **84**, 1895.
18. D. BALASUBRAMANIAN and C. KUMAR, in 'Solution Behaviour of Surfactants', Eds. E. J. FENDLER and K. L. MITTAL, Plenum Press, N. Y. 1981 (in press).
19. S. FRIBERG, Microemulsions—'Theory and Practice', Academic Press, New York, 1977, p. 133.
20. A. D. BANGHAM, M. W. HILL and N. G. A. MILLER, in 'Methods in Membrane Biology', Ed. E. D. KORN, Vol. 1, p. 1, Plenum, New York, 1974.
21. G. GREGORIADIS, in 'Methods in Enzymology', Ed. K. MOSBACH, Vol. XLIV, p. 218, Academic Press, New York, 1976.
22. F. P. GENTILE, F. RICCI, F. PODO and P. E. GNA, *Gazz. Chim. Ital.*, 1976, **106**, 423.
23. N. BLOEMBERGEN, E. M. PURCELL and R. V. POUND, *Phys. Rev.*, 1948, **73**, 679.
24. A. B. MANDAL, S. RAY, A. M. BISWAS and S. P. MOULIK, *J. Phys. Chem.*, 1980, **84**, 856.
25. E. ROTLEVI and A. TREININ, *J. Phys. Chem.*, 1965, **69**, 2645.
26. E. M. KOSOWER, 'An Introduction to Physical Organic Chemistry', Wiley, New York, 1968.
27. D. C. TURNER and L. BRAND, *Biochemistry*, 1968, **7**, 3381.
28. F. M. MENGER and K. YAMADA, *J. Amer. Chem. Soc.*, 1979, **101**, 6731.
29. K. KANO and J. H. FENDLER, *Biochim. Biophys. Acta*, 1978, **509**, 289.
30. M. L. BENDER, F. J. KEZDY and F. C. WEDLER, *J. Chem. Educ.*, 1967, **44**, 84.
31. D. BALASUBRAMANIAN, S. SUBRAMANI and C. KUMAR, *Nature*, 1975, **254**, 252
32. M. NEMAT-GORGANI and G. H. DODD, *Eur. J. Biochem.*, 1977, **74**, 139 and references cited therein.