

Effect of Salt on the Structure of Middle Phase Microemulsions Using the Spin-Label Technique

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The middle phases obtained by varying the sodium chloride concentration in surfactant formulations containing 5:3 (wt/wt) TRS 10-410 (a petroleum sulfonate)-isobutyl alcohol and equal volumes of aqueous and oil phases were studied by using spin-labeling techniques. Two different spin-labels, one partially water soluble (5-doxylstearic acid label) and the other water insoluble (3-doxylcholestone label), were used. Extensive measurements of electrical conductivity and phase volumes of the middle phases were also carried out. These physical property results corroborated the spin-label studies in that below 2.0 wt % NaCl the middle phase was essentially a microemulsion of the water external type. Beyond 2.3% NaCl the appearance of a signal component typical of a free label (ketostearic acid) in an oil environment and changes in correlation time characteristics (cholestone label) coupled with physical property data underlined a qualitative change in the microemulsion system. It is believed that these changes are consistent with a transition from a water-external type to an oil-external type microemulsion system. This transition is estimated to be around 2-2.3% NaCl. The results are further substantiated by ascorbic acid reduction rate studies. Possible mechanisms of this transition are discussed.

Introduction

In our earlier work¹ we investigated the physical property behavior and structural aspects of aqueous solutions of the surfactant system (5.3 wt/wt) TRS 10-410-isobutyl alcohol (IBA) as a function of salt concentration. We also reported a comprehensive study of the sonicated emulsions formed by this system with dodecane oil at varying salinities.²

The phase behavior of this system had heretofore been studied only at room temperature.³ Under these conditions, a 5:3 (wt/wt) TRS 10-410-isobutyl alcohol formulation when equilibrated with dodecane oil exhibits a two-phase pattern up to 1.2% NaCl, while a three-phase behavior is obtained from 1.3 to 2.0% NaCl concentrations. Beyond 2% a reversal to the two-phase behavior occurs. In our experiments¹ on the effect of aging on aqueous surfactant formulations we noted that aged aqueous solutions when equilibrated with equal volumes of dodecane oil generated middle phases at a faster rate and beyond the limit of that for 2% salinity obtained at room temperature. We believe that aging is an essentially physical process producing no chemical changes, and highlights the possibility that equilibration in these systems is achieved over a long period of time. As an alternate to aging we felt that heating these systems might also generate middle phases at a faster rate. We found that equilibration of these mixtures at 65 °C for 24 h gave rise to new phase patterns. The salinity range where a three-phase pattern is observed was significantly enlarged, for example, from 1.3-2% NaCl at room temperature to 1.3-5% NaCl at 65 °C. The middle phases so obtained were stable when left at room temperature (about 23 °C) over a period of at least 10 months.

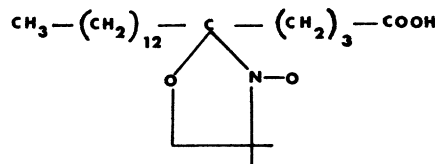
In this paper, we present data on the structure and nature of middle phase microemulsions obtained under the conditions of heat treatment described above, using the spin-labeling technique.

Experimental Section

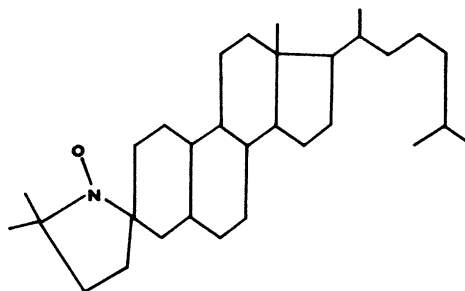
Aqueous solutions of 5:3 (wt/wt) TRS 10-410-isobutyl alcohol of appropriate salinity, where the salt concentration

is based on weight percentage sodium chloride against weight of water, were prepared and an equal volume of dodecane oil was added. The systems were gently mixed and equilibrated at 65 °C for 24 h. The choice of temperature and of period of equilibration was based on a series of experiments on the rate of equilibration at different temperatures. At lower temperatures, e.g., 35 and 45 °C, equilibration was slower and not practical. We chose 65 °C since equilibration was faster at this temperature and yet was not too high for decomposition or loss of components to occur. Equilibration at 65 °C for periods of greater than 24 h did not alter the middle phase patterns. All mixtures were equilibrated in cylinders with stoppers to prevent evaporation losses.

The spin labels 5-doxylstearic acid (I) and 3-doxylcho-



LABEL I : 5-DOXYL STEARIC ACID



LABEL II : 3-DOXYL CHOLESTONE

lestane (II) were used in this study. I was dissolved in the (5:3 wt/wt) mixture of TRS 10-410-IBA and II in dodecane oil before equilibration with appropriate phases. The

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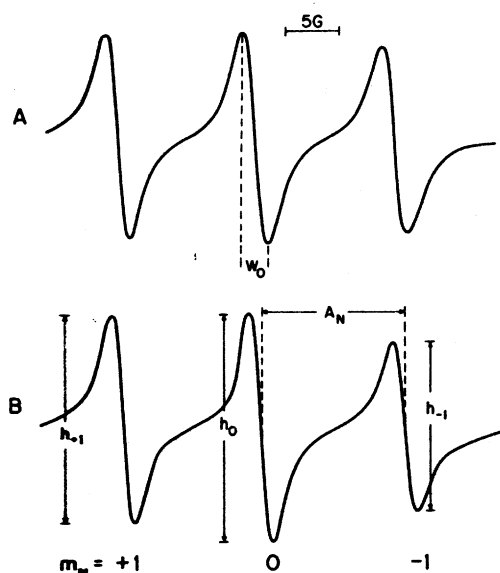


Figure 1. ESR spectra of II in (A) dodecane oil and (B) 1.8% NaCl, middle phase microemulsion.

concentrations of the labels were maintained so as to be around 2×10^{-4} M in the mixture.

All ESR spectra were recorded by using an E-9 spectrometer operating at a microwave frequency of 9.5 GHz and a modulation frequency of 100 kHz. Modulation amplitude was carefully chosen to avoid line broadening artifacts. Scan ranges were 100 and 10 G for order parameter and line width determinations for label I systems and 40 and 4 G for hyperfine coupling constant and correlation time determinations for label II systems. For line width determinations each manifold was individually recorded. The scan rate was about 1 G/min for such measurements. Ambient temperature was 23 ± 1 °C. A minimum of two scans were employed for a single system.

TRS 10-410 was supplied by Witco Chemical Co. and used as received. All other chemicals were Analar grade or better. Dodecane was procured from Fischer Scientific Co. and was of greater than 99% purity. Water used was double distilled and passed through an ion-exchange column. Degassing of samples for removal of oxygen was not attempted since foaming had adverse effects on these systems.

Ascorbic acid treatment was carried out by using solid ascorbic acid since addition of ascorbic acid aqueous solutions alters the middle phase compositions. Ascorbic acid was preweighed in the ESR sample tubes and the desired solutions containing I was introduced carefully with a syringe just prior to measurements. The kinetics of reduction of the label was followed by obtaining the ESR spectra at different time intervals. After each spectral run the recorder pen was vertically displaced for the next measurement. The ascorbic acid concentration in all solutions was around 10^{-2} M.

The spin labels were purchased from Syva, Palo Alto, Calif., and used as received.

Location of Probes

Cholestane Label. Figure 1 shows typical ESR spectra of the cholestane label (II) for two different cases. The motion of the label is isotropic in nature in both cases, as evidenced by the appearance of three well-defined and sharp lines. The hyperfine coupling constants and the correlation time for rotational tumbling motion are indicative of the microenvironment in such cases. The hyperfine coupling constant, A_N , which is 13.2 G in all the

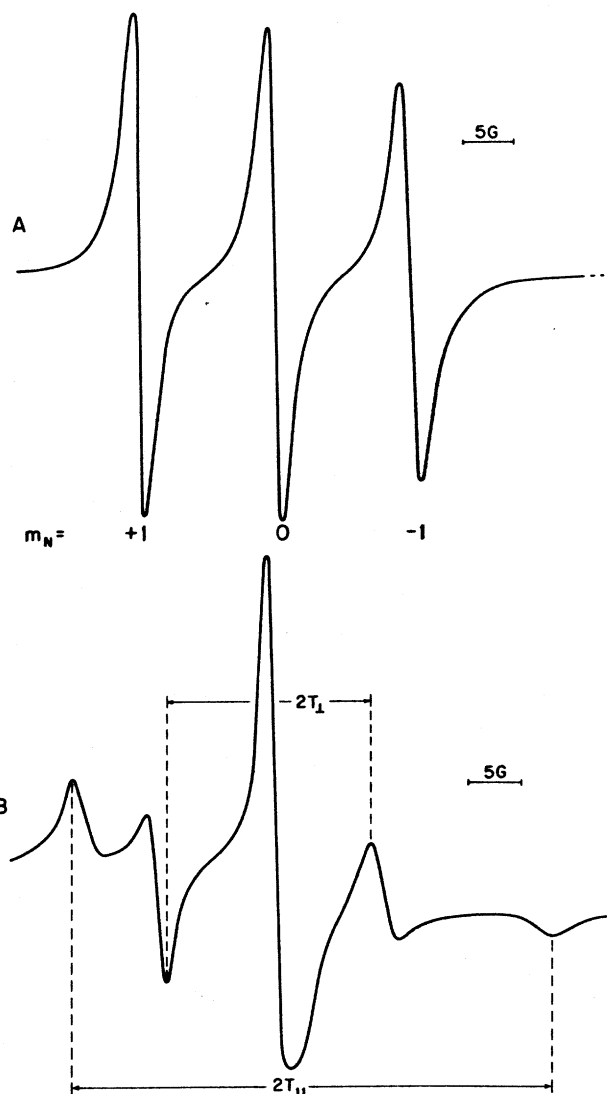


Figure 2. ESR spectra of I in dodecane oil (A) and 1.4% NaCl (B) middle phase microemulsion.

systems studied using II, shows that the label resides exclusively in an oil environment. Butler et al.⁴ have demonstrated that the cholestane label might be excluded totally from a rigid phase if a more fluid phase coexists along with the rigid phase. Intercalation of II at the oil/water interface would attribute anisotropic motional characteristics to the label.⁵ However, for the cholestane label so intercalated there are orientations wherein a three-line spectrum could arise. If the field axis is perpendicular to the interface, it is parallel to the nitroxide radical y axis for the cholestane label. Since motion about the y axis is isotropic a three-line spectrum would be obtained. However, the hyperfine coupling constant would be less than 10 G (around 6 G for oriented bilayers⁵). The other possibility is that there is total disorder at the interface. This implies that the label has freedom of motion about all three axes. In such a situation the hyperfine coupling constant is an average of the values in the three directions. For the cholestane label this is around 15 G in bilayers.⁶ Our experiments with the 5-doxytstearic acid label show that there exists a high degree of order in the interfacial region. It is known that for bilayers this ordering is higher as we approach the interface.⁶ Therefore, the notion of complete disorder in the interfacial region has to be discarded.

In effect, the A_N value of 13.2 G for the cholestane label in the middle phases as well as in pure oil combined with order parameter determinations from other label studies, proves that the cholestane label prefers the oil phase to intercalation at the interface (Figure 1).

Stearic Acid Label. Figure 2A shows the spectrum of stearic acid label (I) in dodecane oil. Such three-line spectra are obtained whenever the fatty acid chain is not anchored strongly at the oil/water interface via the carboxylate group. Figure 2B shows the spectrum of the spin-labeled stearic acid in a middle phase microemulsion system. It is immediately noticed that the spectrum has fairly well-defined extrema. This spectrum is anisotropic in nature. Similar spectra have been obtained with stearic acid labels for spin-labeled phospholipid dispersions,⁸ egg lecithin vesicles,⁹ and normal sarcoplasmic vesicles.¹⁰ The observed shape of such spectra has been identified with that arising from axial symmetry of nitroxide group motional characteristics. This situation arises mainly due to a strong anchoring of the fatty acid chain through the carboxyl group at the oil/water interface.

Theory

Correlation Time for Reorientation of the Tumbling Probe, τ_θ . If the label tumbles rapidly in solution so that during one oscillation of the microwave field it can assume many orientations relative to the static magnetic field, then the anisotropic dipolar interaction is averaged to zero. Consequently, the observed spectrum is entirely due to isotropic coupling. This rotational motion of the label is characterized by a correlation time τ_θ , defined as the time it takes the label to rotate through an angle of 1 rad.

For isotropic motion of the label, the line widths are almost entirely due to motional effects. Kivelson's theory (see, for example, ref 7) expresses the line widths in terms of coefficients that are related to the rotational correlation time, τ_θ . Hence from the experimental line widths one can calculate τ_θ , provided the condition of rapid tumbling motion of the label is satisfied. This condition is met when $\omega^2 \tau_\theta^2 \ll 1$, where ω is the microwave frequency. Thus for 9.5-GHz microwave frequency, τ_θ has to be less than 10^{-9} s for Kivelson's theory to be valid.

In our systems, τ_θ was calculated by using eq 1, derived

$$\tau_\theta = (6.5 \times 10^{-10}) W_0 \left[\left(\frac{h_0}{h_{-1}} \right)^{1/2} + \left(\frac{h_0}{h_{+1}} \right)^{1/2} - 2 \right] \text{ s} \quad (1)$$

by Stone et al.,⁷ where h_0 is the height of central ($M_N = 0$) line, h_{-1} is the height of high field ($M_N = -1$) line, h_{+1} is the height of low field ($M_N = +1$) line, and W_0 is the line width of central line (Figure 1).

The τ_θ values so calculated are of the order of 10^{-10} s. Therefore, the use of Kivelson's theory and hence eq 1 to obtain relative changes in the fluidity of the label in the microenvironment is justified.

The correlation time is directly proportional to the viscosity of the medium since the reorientation is more difficult in viscous media. Thus, τ_θ is an excellent monitor of the viscosity of the label environment. Conversely, changes in viscosity can affect τ_θ significantly.

Anisotropic Spectra and Order Parameters. The theory associated with the motion of fatty acid labels in membranes, vesicles, and dispersions is well documented.^{6,8-15} There are two types of molecular motion that the nitroxide group of the spin-labeled fatty acid can undergo. Each carbon-carbon segment of the fatty acid chain can have independent motion, termed flexing mobility, and in saturated chains this mobility is known to be high. As the

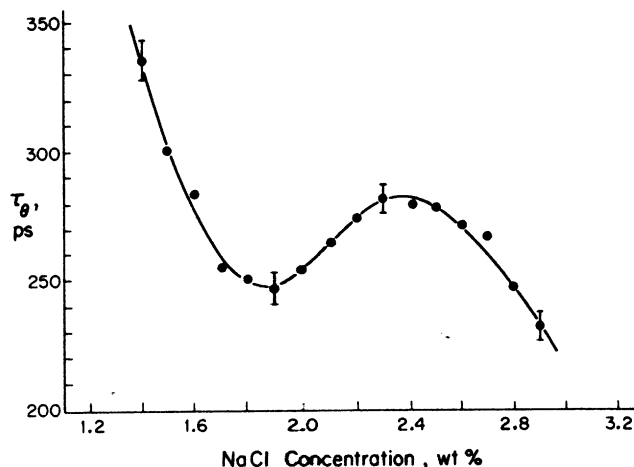


Figure 3. Variation of correlation time for rotational tumbling motion, τ_θ , of II in middle phase microemulsions at different salinities.

nitroxide group is moved toward the carboxyl group, the mobility decreases indicating a more rigid state of the chain near the interface.⁶ A second type of molecular motion for the nitroxide moiety is a rapid rotation about the long molecular or chain axis. The spin probe long axis precesses rapidly around the normal to the interface and describes a cone. The $2p\pi$ orbital of the nitrogen of the nitroxide group makes an angle β with the normal and this angle denotes the deviation from normal. An increase in β indicates a less ordered fatty acid chain. The rapid anisotropic motion about the molecular axis imparts axial symmetry giving rise to two modes of interaction between the spin and the external magnetic field. The observed splittings with the applied field parallel to the axis of rotation is denoted by $T_{||}$ and that in the perpendicular direction T_{\perp} (Figure 2B). The quantity $T_{||} - T_{\perp} = \Delta T$, is an indirect measure of the order in the system. The order parameter S is given by

$$S = (T_{||} - T_{\perp}) / (T_{zz} - T_{xx})$$

where T_{zz} and T_{xx} are the splittings when the spin label is oriented parallel and perpendicular, respectively, to the magnetic field. The values for T_{zz} and T_{xx} for the spin label used here were taken from the literature.¹² The order parameter S is related to the angle of deviation, β , through the expression

$$S = (1/2)(3 \cos^2 \beta - 1)$$

Therefore, a decrease in order parameter, S , denotes an increase in deviation of the rotational axis from the normal. An increase in angle of deviation β is an indication of increased mobility of the chains.

It is seen therefore that fatty acid labels undergo fast anisotropic motion about the molecular axis in addition to a flexing motion. Spectra with a high degree of anisotropy and well-defined extrema are obtained (Figure 2B). If the amplitude of motion of the spin-label is increased and coupled with a decrease in the rate of rotation, the anisotropy disappears and isotropic three-line spectra are obtained. The flexing motion of the chains in these cases are enhanced making the chains more mobile.

Results

Figure 3 shows the variation of correlation time τ_θ of II as a function of salinity in the middle phase microemulsions. τ_θ decreases rapidly initially and goes through a

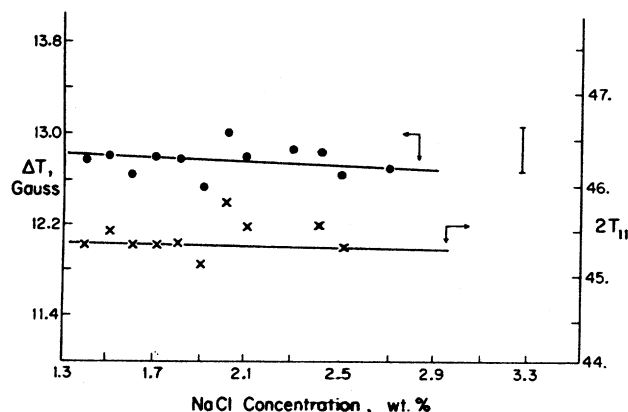


Figure 4. Variation of order parameter, ΔT , and separation between outer extrema, $2T_{II}$, of I in middle phase microemulsions at different salinities.

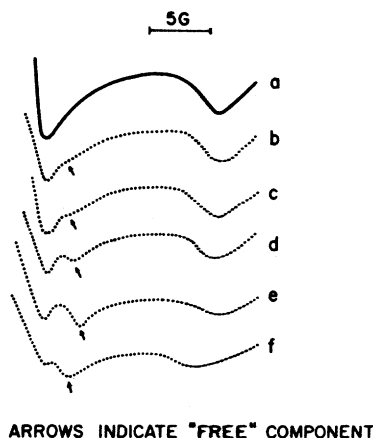


Figure 5. High-field extrema of spectra of I in various systems: 1.9% (a), 2.0% (b), 2.1% (c), 2.4% (d), and 2.7% (e) NaCl middle phase microemulsions; (f) 8.0% NaCl oil phase.

minimum around 2.0% NaCl. Beyond 2% NaCl, τ_θ increases gradually and exhibits a maximum around 2.3% NaCl. τ_θ decreases beyond 2.5% NaCl, reaching values comparable to those obtained in oil phases.

Figure 4 shows the variation of order parameters ΔT and $2T_{II}$ of I in the middle phases as a function of salinity. There are no significant changes in either of these parameters, indicating an essentially constant packing at the interface and mobility of the chains. The nature of the spectra, however, show interesting changes in line shapes and we will discuss them in detail. Figure 5 shows the high-field extrema from spectra of I in the middle phases as a function of salinity. For comparison, spectra of I in oil phases containing most of the surfactant are shown. It is seen that beyond 2% salinity a new signal component appears. As salinity increases this component increases in intensity.

Figure 6 shows the change in line height of the central line, h_0 , as a function of time for I in various systems treated with ascorbic acid. The central line height could include a contribution from the ascorbate radical spectrum. The decay of the low-field line intensity, which lies outside the range of the ascorbate radical, could not be followed due to changes in line shape with time. The first-order decay of the spectra as followed by the central line justifies use of decay of the central line intensity. Hence, the rate of decay of h_0 only is known. Table I shows the time $t_{1/2}$ from the start of the signal to 50% decay. The initial h_0 values are not the same since the concentration of the label in the various phases are different although the total label

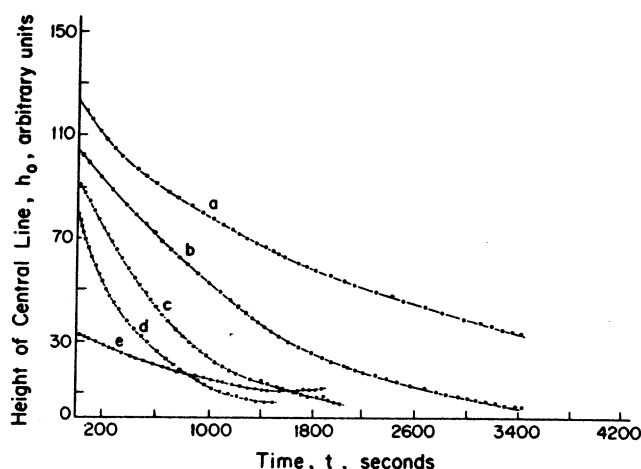


Figure 6. Plots of line height of central line, $M_N = 0$, of ESR spectra of I in various systems treated with 10^{-2} M ascorbic acid: (a) 1.4% NaCl middle phase microemulsion, (b) 0.5% NaCl aqueous phase, (c) 2.7% NaCl middle phase microemulsion, (d) dodecane oil, (e) 3.0% NaCl oil phase.

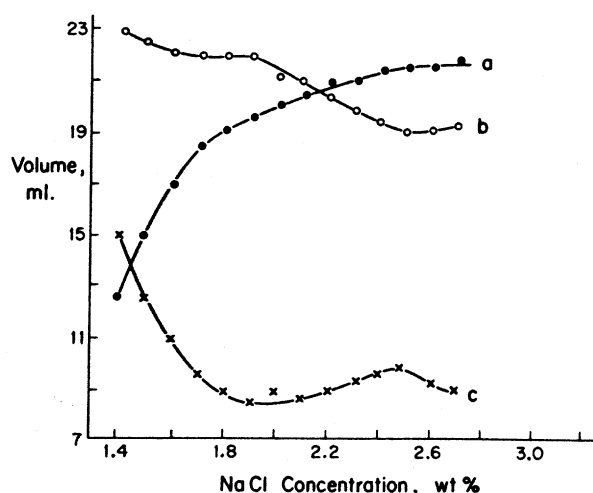


Figure 7. Plots of volumes of different phases as a function of salinity: (a) aqueous phase, (b) oil phase, (c) middle phase.

TABLE I: Ascorbic Acid Reduction Rate Studies

system	$t_{1/2}$, s
water	< 60
0.5% NaCl, aqueous phase	1040
1.4% NaCl, middle phase	1600
2.7% NaCl, middle phase	560
3.0% NaCl, oil phase	700
dodecane oil	600

concentration in the mixture was kept constant.

Conductivity studies indicated that as the salinity increases there is a steady decrease in conductivity of middle phases. Beyond 2% NaCl, the conductivity values are essentially less than $1 \mu\text{S}/\text{cm}$, comparable to values typical of an oil environment. Phase boundary behavior patterns are helpful indications of the type of microemulsion present. At low salinities the aqueous phase-middle phase boundaries are blurred and the oil phase-middle phase boundaries are clear. For salinities above 2% NaCl the reverse is observed: the oil phase-middle phase boundaries are blurred while the middle phase-aqueous phase boundaries are clear. Figure 7 shows a plot of the volumes of various phases as a function of salinity. It is noticed from Figure 7 that beyond 2.3% NaCl the aqueous phase

volume is almost constant. It is reasonable to assume that the middle phases above 2.3% NaCl are derived from the oil phase.

Discussion

Correlation Time, τ_θ , Studies of II. The high values of τ_θ at lower salinities (1.3% NaCl) indicate a high restriction to motion of the label. The decreasing trend in τ_θ as salinity increases reveals that this restriction is gradually removed. If the cholestane label is intercalated at the surface of water-external microemulsion, τ_θ would have either shown an increase as the salinity increases, since the microemulsion would be larger or been constant since the packing at the interface is unchanged (Figure 4).

Bulk viscosity correction for τ_θ cannot be applied since the label is sensitive only to the viscosity of the label microenvironment. The cholestane label is located in the oil phase at all salinities. We have already shown² that the correlation time τ_θ of the cholestane label in oil phases containing trace amounts of surfactant and alcohol is around 100 ps. It was also shown that τ_θ for surfactant-rich oil phases was around 170–180 ps. Hence, the high values of τ_θ , typically 250–350 ps, for salinities below 2% NaCl and between 2 and 2.5% NaCl cannot be accounted for by viscosity changes that might be brought about by surfactant and/or alcohol dissolution in the oil core. Therefore, the high values of τ_θ arise due to factors other than viscosity. The decreasing trend in τ_θ values is consistent with a water-external microemulsion in which the label resides in the oil phase. With increasing salinity, the core volume, or oil volume, increases, facilitating motional freedom of the label. The minimum in τ_θ around 2% NaCl and the subsequent increase beyond 2% NaCl highlights the achievement of an optimum core volume and a possible qualitative change in the nature of the microemulsion. The constancy of the A_N values in the entire range supports the view that the cholestane label reports only the changes in the oil phase of the microemulsion. We shall discuss the region where τ_θ increases, 2–2.3%, later. However, it is felt that this region comprises a set of processes that ultimately results in phase-inversion leading to the complex τ_θ behavior.

Beyond 2.3% NaCl, τ_θ decreases, indicating an environment that facilitates label rotational motion. The values of τ_θ are typical of the label in an oil phase rich in surfactant. An oil-external microemulsion configuration could give rise to such a τ_θ behavior as the label is located in the external oil phase. Increasing salinity decreases the core volume and increases the oil-external phase volume, making the environment less rigid to label motion. The decreasing trend in τ_θ , along with other results, notably conductivity values of the order of 0.5 $\mu\text{S}/\text{cm}$ and less, suggest an oil-external type configuration for the microemulsion.

Examining the data from spin-label stearic acid middle phases, we observe that the interfacial packing and chain mobility of the microemulsions is unchanged over the entire middle phase range. Thus we can rule out significant changes in composition of surfactant and alcohol at the oil/water interface that might lead to the observed change in τ_θ behavior. It is reasonable to assume that restrictions on the cholestane label rotational freedom are a direct consequence of the type of microemulsion present as well as changes in core volume due to a change in solubilization capacity as a function of salinity.

Spectral Features of the Stearic Acid Label in the Middle Phases. Close examination of Figure 5 reveals that a new component appears beyond 2% salinity. This component has been characterized as that due to "free"

label in an oil environment, based on an A_N value of 13.2 G for I in dodecane. Systems exhibiting similar behavior have been studied with stearic acid labels.^{4,14} As salinity increases the "free" label component increases and at 2.7% NaCl it is very similar to that of I in a 8% NaCl oil phase system. There are two mechanisms for this process: As salinity increases from 1.3 to 2%, the oil core volume of the water-external microemulsion increases. This increase in microemulsion size has to be stabilized by an incorporation of additional surfactant molecules at the interface. Hence the core volume can increase only as long as surfactant molecules are available for incorporation at the interface. The appearance of free surfactant molecules in an oil phase indicates that the surfactant molecules are released from the interface. We have shown² that only trace amounts of surfactant are present in the aqueous and oil phases. Thus almost all the surfactant molecules are in the middle phase. If an increase in salinity releases surfactant molecules into the oil environment of the middle phase microemulsion it signals, (1) a reduction in core volume of the microemulsion and/or (2) a change in the type of microemulsion. (1) A reduction in core volume and microemulsion drop size entails a smaller surfactant population needed for stabilization of the interface. Surfactant molecules are consequently released into an oil environment since the salinity in the aqueous environment precludes hosting the surfactant monomers. This mechanism does not require an inversion. (2) An alternate mechanism would encompass a change in the type of microemulsion, from a water-external one to an oil-external type. This once again entails a redistribution process and results in the release of surfactant molecules into the oil environment. For a microemulsion of the oil-external type, increasing salinity decreases the core volume and the surfactant population required at the interface steadily decreases. As stated earlier the high salinity of the aqueous environment forces the released surfactant monomers to seek an oil environment. The second mechanism necessitates a transition around 2% NaCl.

Both the above processes could be occurring in the region beyond 2% NaCl. If mechanism 1 is operative in the region beyond 2% NaCl, the cholestane label motion would be gradually more restricted for a water-external microemulsion configuration as the salinity increases. However, τ_θ increases in the region (2–2.3% NaCl) and subsequently decreases beyond 2.3% NaCl. Thus mechanism 1 could be the dominant process in 2–2.3% NaCl but not beyond 2.3% NaCl.

On the other hand, if mechanism 2 is operative in the region beyond 2% NaCl, the label motion would depend on several factors. Around 2% NaCl, inversion from water-external to an oil-external configuration is predicted by this mechanism. It is reasonable then to expect a maximum or an optimum core volume at the inversion salinity. Increasing salinity would decrease water core volume and, since the label is in the oil phase, τ_θ should progressively decrease. Thus, mechanism 2 appears to be dominant beyond 2.3% NaCl.

The two mechanisms therefore predict the following: Mechanism 1 predicts that τ_θ increase for the 2–2.3% NaCl range and further increase from 2.3 to 2.8% NaCl and beyond. Mechanism 2 predicts that τ_θ decrease for the 2–2.3% NaCl range and a further decrease from 2.3 to 2.8% NaCl and beyond.

It appears as though mechanism 1 dominates in the 2–2.3% NaCl range and mechanism 2 dominates beyond 2.3% NaCl. However, since it is not possible to ascertain size and number factors, it is probable that both mecha-

nisms operate in the 2–2.3% salinity range. Beyond 2.3% NaCl mechanism 2 is the only one that is compatible with the experimental results.

Ascorbic Acid Reduction. Rate Studies

In order to ascertain the validity of the above two processes (1 and 2) microemulsions of four representative systems, 0.5% aqueous phase, 1.4% middle phase, 2.7% middle phase, and 3% oil phase, along with pure water and pure oil containing I were treated with ascorbic acid. From Figure 6 the time required for the signal to decay by 50% can be obtained. These values are presented in Table I.

In the past, ascorbic acid reduction studies have been carried out by a number of investigators;^{16–19} Paleos and Dais¹⁶ have studied the ascorbic acid reduction of nitroxide to a diamagnetic hydroxylamine. Ascorbic acid reduction has been employed to study flip-flop rates in phospholipid dispersions¹⁷ and other related systems.¹⁸ An excellent discussion on the advantages this treatment offers in monitoring the location and permeability characteristics in membranes is presented by Schreier-Muccillo et al.¹⁹

In our study, I was instantly reduced in pure water. The reduction rate was much slower in oil. Dissolution of ascorbic acid at the solid/solution interface followed by rapid diffusion into solution with concomitant label reduction is indicated for aqueous solutions. On the other hand, since ascorbic acid does not dissolve in the oil, diffusion of solution containing I into the solid is the only process that could bring the label and the ascorbic acid together. The reduction of the label is assumed to be rapid since the polarity of the medium does not affect the reaction rate.¹⁹

Reoxidation of the spin-label by molecular oxygen (see ref 17) could complicate the kinetics of reduction of the label by ascorbic acid, if an appreciable amount of the reduced-label spectrum is restored. No evidence of reoxidation was observed in the first-order decay of the spin-label spectrum. Reoxidation of the label was observed by Kornberg and McConnell¹⁷ for aged preparations of lecithin vesicles over extended periods of time at 45 °C. In our system, reoxidation is assumed to be unimportant since (1) all solutions were fresh and (2) the time scale of the experiments were much shorter (less than 1 h). Therefore, we believe that the rate of reduction of the label by ascorbic acid is due to the diffusion processes outlined earlier.

For I in 0.5% aqueous phase and 1.4% middle phase, the immobile component corresponding to the interfacial label persists for over 20 min. Also the decay of this component occurs at the same rate as that for other signal components. In the 2.7% middle phase and the 3% oil phase systems, the "immobile" interfacial component is destroyed completely within 3 min. The rate of signal decay is much faster than in the 0.5 and 1.4% systems. It is also seen that this rate is comparable to the rate of reduction of the label in a pure oil environment.

It is observed that for the 0.5% aqueous and 1.4% middle phases the $t_{1/2}$ values are similar. It is also observed that the 2.7% middle phase and 3% surfactant-rich oil phase show $t_{1/2}$ values that are close to those obtained in dodecane oil (Table I). We can divide these systems into two sets: (set 1) 0.5% aqueous and 1.4% middle phases and (set 2) 2.7% middle and 3% oil phases, and pure oil phase. The similarity of $t_{1/2}$ values of the systems in each set reveals that the rate-governing process is similar for systems of that set, and is a significant pointer of the label accessibility to ascorbic acid reduction.

For set 1, the label reduction is relatively slow. This implies that the nitroxide label is not easily accessible for reduction by ascorbic acid. A protected nitroxide moiety

is suggested. For I the nitroxide group is five carbons away from the head group. In a water-external configuration, the nitroxide moiety would be located within the internal oil core, surrounded by the external water phase. Dissolution of ascorbic acid in the external phase is followed by rapid diffusion to the oil/water interface. If the label is located in the external phase it would be instantly reduced. However, the high $t_{1/2}$ values and the persistence of the "immobile" component rules out this possibility. Hence the slow reduction of the label is due to the direct diffusion of ascorbic acid across the oil/water interface, which is either minimal or absent, and to the label exchanging between the internal and external phases. The results, therefore, strongly support the view that set I microemulsion systems have the water-external type configuration.

For set 2 systems, $t_{1/2}$ values are distinctly different from set 1 systems, highlighting a different process of label reduction by ascorbic acid. This process appears to be the diffusion of the solution across the solid/solution interface as seen from the similarity with the pure oil system. This imparts oillike characteristics for the 2.7% middle phase and 3% surfactant-rich oil phase. It is reasonable to deduce that, in these systems, oil is the external phase. For an oil-external configuration, the nitroxide moiety is now located in the external oil phase. Reduction of the nitroxide would occur when diffusion of the solution brings it in contact with ascorbic acid. Ascorbic acid diffusion across the oil/water interface is rapid and is favored. The "immobile" interfacial signal component is thus destroyed at much shorter time periods. The closeness of these time periods to the $t_{1/2}$ value in the pure oil system argues very strongly for an oil-external type configuration. It is clear that for 2.7% middle phase and 3% oil phase, an oil-external-type microemulsion is the most probable configuration.

Conclusions

(1) The surfactant formulation containing 5:3 (wt/wt) TRS 10-410-isobutyl alcohol and sodium chloride in water and an equal volume of dodecane oil produces three equilibrium phases at elevated temperatures in the salinity range 1.3–3.5 wt %.

(2) The middle phases obtained by heat treatment at 65 °C for 24 h are stable when allowed to stand at room temperature.

(3) Middle phase microemulsions produced by preheating the aqueous-oil system exhibit an inversion process, from a water-external type to an oil-external type, upon increasing salinity.

(4) Changes in spectral features, correlation time behavior, rates of reduction of labels, electrical conductivity, and phase volume behavior all confirm such an inversion process.

(5) The range of salinity wherein inversion occurs has been estimated to be around 2–2.3 wt % NaCl in these systems. The inversion process appears to be a smooth transition and the possibility of two types of microemulsions coexisting in this range is not ruled out.

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