

## HIGH RESOLUTION NMR (220 Mc) STUDIES ON THE STRUCTURE OF WATER IN MICROEMULSIONS AND LIQUID CRYSTALS \*

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### INTRODUCTION

Microemulsions are optically clear, stable dispersions of oil and water usually obtained by using a variety of surface-active molecules or mixtures of molecules as emulsifiers.<sup>1,2</sup> Such oil-in-water or water-in-oil microemulsions have been examined by low-angle x-ray measurements,<sup>3</sup> light-scattering techniques,<sup>4</sup> ultracentrifugation,<sup>5</sup> electron microscopy,<sup>2</sup> and viscosity measurements,<sup>6</sup> and have been shown to consist of droplets 100-600 Å in diameter. The water-in-oil type of microemulsion and the liquid crystalline phases provide useful systems for the study of the structure of water near charged surfaces produced by polar groups of emulsifiers. In this paper I report the effect of an increase in the water content of microemulsions and of liquid crystals on their electrical, optical, and nuclear magnetic resonance (NMR) characteristics.

It is generally believed that the clinical potency of anesthetics is related to their solubility in lipids. However, the changes in orientation of lipid molecules and in interfacial forces that can be brought about by the solubility of anesthetics in membrane lipids have not been delineated. This paper reports the use of liquid crystalline structures as a model membrane for studying the effect of  $Ca^{++}$  and local anesthetics (nupercaine, tetracaine, cocaine, procaine) on the interfacial forces and orientation of lipid molecules.

### MATERIALS AND METHODS

Hexadecane and hexanol of purity >99% were obtained from Chemical Samples Co. (Columbus, Ohio), and purified oleic acid was obtained from Mann Research Laboratories (New York, N.Y.). Double distilled water and inorganic chemicals of reagent grade were used throughout the work.

Microemulsions were produced by mixing hexadecane (oil), hexanol, oleic acid, and potassium hydroxide in a test tube in the following proportions: for 1 ml of hexadecane, 0.4 ml of hexanol and 0.2 g of potassium oleate (i.e., the necessary amounts of oleic acid and KOH) were added. Water was added in small amounts to this mixture, which was then shaken vigorously. Optical clarity, birefringence, electrical resistance, and NMR measurements were made upon the gradual addition of water to this mixture. Two polarizing plastic sheets were arranged perpendicular to one another to detect birefringence of

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the microemulsions. Two glass-sealed copper wires, 0.16 cm thick, were used as electrodes. A 1-cm length at the end of each wire was exposed outside the glass tube, and the copper wires were separated by 0.8 cm. The electrical resistance of the mixture was measured by dipping the electrodes, connected to a conductivity bridge (Beckman model RC 16B2), into the microemulsions. NMR spectra were obtained after the gradual addition of water to the microemulsion in the sample tube of the NMR spectrometer (Varian HA-220 megacycle). Tetramethylsilane was used as an internal standard.

To study the effect of anesthetics and Car, the birefringent phase occurring at the water/oil ratio 1.2 to 1.4 was used. Dry crystals of procaine·HCl, cocaine·HCl, tetracaine·HCl, and nupercaine·HCl were added separately in increasing amounts to a measured volume of the liquid-crystalline phase. After addition of anesthetics the mixture was vigorously stirred with a glass rod and the electrical resistance and birefringence were measured.

### RESULTS AND DISCUSSION

FIGURE 1 shows the optical and birefringence characteristics as well as the measured electrical resistances as a function of the water content of the microemulsions.

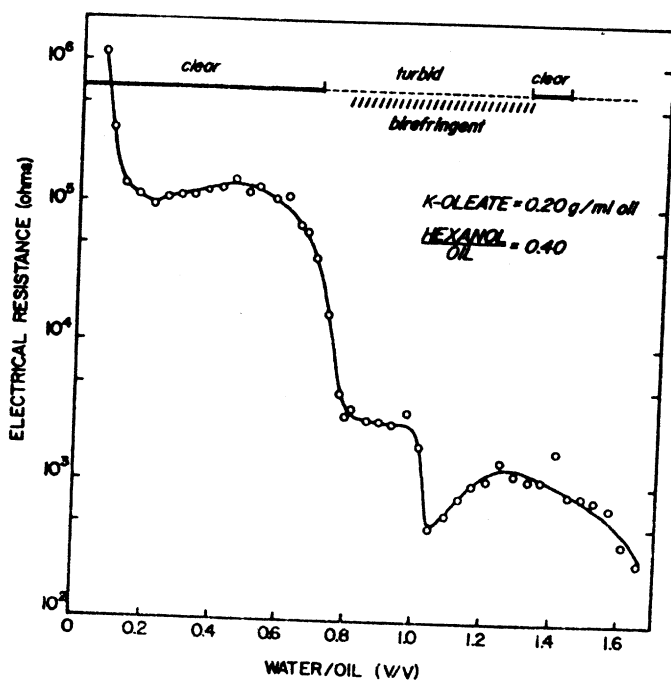


FIGURE 1. Variation in electrical resistance, optical clarity, and birefringence of microemulsions as the water content increases. The microemulsion contains 0.2 g of potassium oleate per ml of oil; the ratio of hexanol to oil is 0.4 by volume.

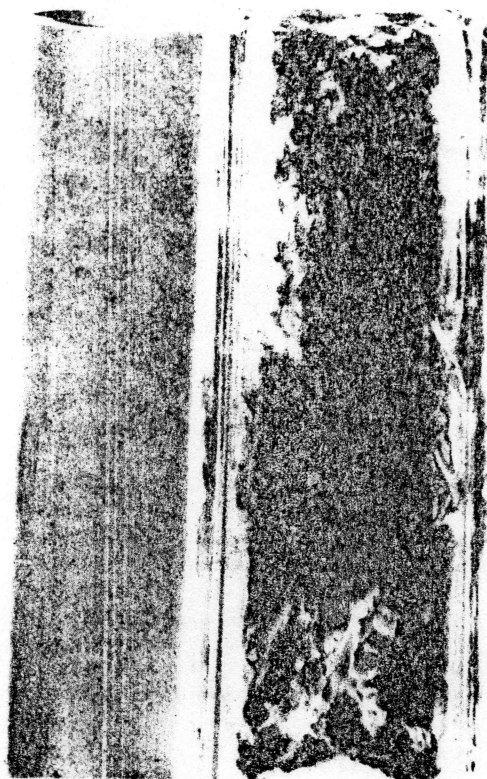


FIGURE 2. The dispersions from the two clear regions and the turbid region were placed between polarizing plastic sheets; only the dispersion from the turbid region exhibited birefringence (middle bottle). The dispersions in the clear regions were isotropic.

FIGURE 2 shows the appearance of birefringence in the dispersions from the turbid region. The dispersions in the two clear regions were isotropic (samples on both sides of the birefringent sample in FIGURE 2).

FIGURE 3 shows the chemical shifts and bandwidths at half height of the major peaks in the NMR spectra of the microemulsions. As the amount of water increases, the microemulsion passes through a clear to turbid to clear transition. In contrast to the two clear regions, the turbid region exhibits birefringence. After clarity returns for the second time, the dispersion becomes opaque, milky, and nonbirefringent (FIGURE 1). The variation of the electrical resistance as a function of water content follows an unusual pattern. For ratios of water to oil from 0.2 to 0.6, there is no significant change in the resistance. In the birefringent region, however, the electrical resistance falls sharply at ratios close to 0.7 and 1.0, and subsequently it increases and then decreases (FIGURE 1). The NMR data also indicate that in the birefringent region distinct

changes occur in the chemical shifts and in the broadening of resonance peaks of both water and hydrocarbon protons. It is evident that the chemical shift of water protons is markedly influenced in contrast to that of methylene or methyl protons (FIGURE 3).

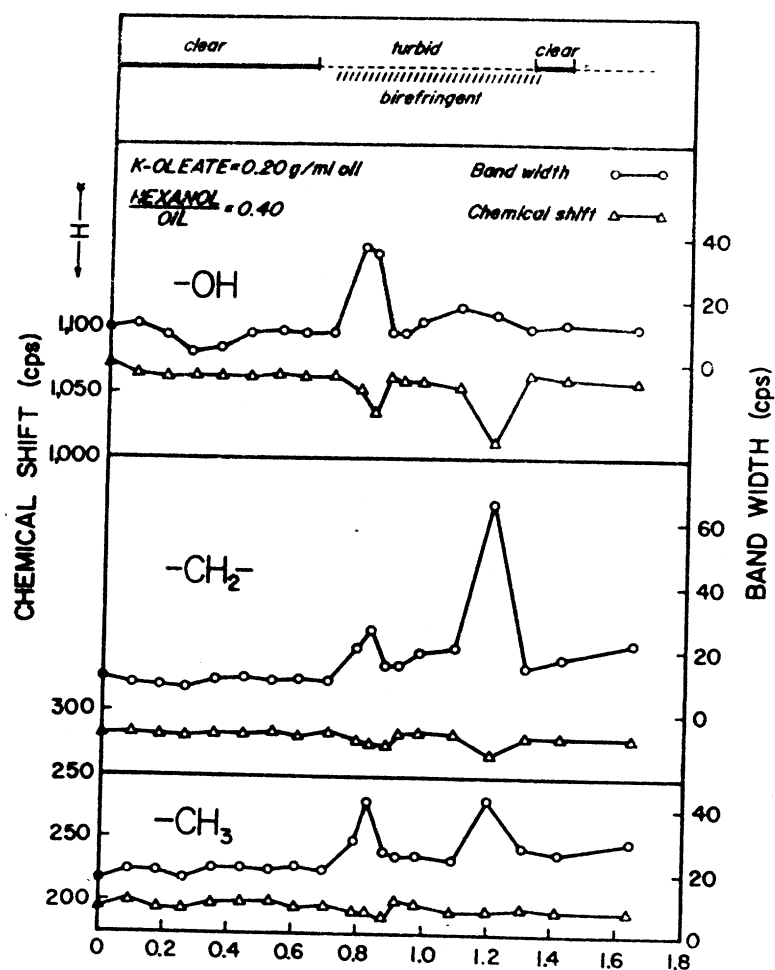


FIGURE 3. Variation in the bandwidth at half height and chemical shift of water, methylene, and methyl protons in high resolution NMR (220 Mc) spectra of the same microemulsions as that in FIGURE 1, as the water content increases. The upper part of the diagram shows corresponding data on optical clarity and birefringence.

The variation in electrical resistance is explained as follows. The resistance drops from  $10^6$  to  $10^5$  ohm as the ratio of water to oil approaches 0.1; this effect is presumably due to the molecular solubilization of water in the hexadecane-hexanol-potassium oleate mixture. This interpretation is supported by

the observation that the hydroxylic protons of hexanol show an upfield shift from 1075–1065 Hz with the initial addition of water. The occurrence of a single peak for hydroxylic and water protons suggests a rapid rate of exchange between these protons. The constancy of resistance between the ratios 0.1 to 0.65 suggests that further addition of water results in the formation of microemulsions consisting of water spheres in the continuous oil medium, in which the interface between the oil and water is the main barrier controlling the ion transport between the electrodes. It is expected that microemulsions having such water spheres would be nonbirefringent.

An abrupt drop in resistance in the birefringent region cannot be explained in terms of an abrupt increase in the dissociation of potassium oleate since this does not explain the development of birefringence. The sharp drop in resistance and the development of birefringence are due to a transition in the structure of water from water spheres to water cylinders to water lamellae in this system. The NMR data, which indicate that water exists in two distinct molecular environments in the birefringent region, support this mechanism. In the first environment in the birefringent region the chemical shift of water protons moves upfield by 25 Hz, and in the second environment by 50 Hz compared with that of water spheres (FIGURE 3). However, the bandwidth at half height of water protons is considerably greater in the first environment than it is in the second environment. The bandwidth is related to the molecular mobility or motion. In general, the greater the bandwidth, the smaller the molecular mobility. The measurements of the bandwidth at half height suggest that water molecules have less mobility in the first environment than in the second. By contrast with water protons, the bandwidth of methylene protons suggests that hydrocarbon chains are less mobile in the second environment than in the first. These characteristics are expected if the first environment consists of water cylinders dispersed in a continuous oil medium and the second environment consists of water and oil lamellae. Water molecules would be less mobile in the cylinders than in the lamellae. Moreover, the formation of these structures would also decrease electrical resistance since the ions could migrate within water cylinders or lamellae without passing through the oil-water interface. The formation of these structures can also account for the development of birefringence. The ratios of water to oil characterizing two distinct molecular environments on the basis of NMR data are slightly higher than those showing an abrupt decrease in electrical resistance. This effect is presumably due to the high spinning rate (6,500 rpm) of the NMR sample tube, which may provide enough mechanical energy to shift the transition of water spheres to water cylinders, causing a higher ratio of water to oil.

The most interesting finding is the observation that the chemical shift of water when it is distributed as spheres is different from that of water distributed as cylinders or lamellae. It can be suggested from data on upfield chemical shifts of water protons (FIGURE 3) that the energy required to flip the proton spin occurs in the following order: water spheres < water cylinders < water lamellae. This ordering is of considerable importance in understanding the functioning of water in membranes and other biological systems.

The existence of such water cylinders of diameter 10–35 Å and lamellae 5–30 Å thick has been established in various lipid-water systems.<sup>7</sup> Zlochowicz and Schulman<sup>8</sup> have shown that tight packing of hydrocarbon chains in the lamellar structure causes extreme broadening of the methylene peak in the NMR spectra. The molecular environments of these water cylinders and

lamellae are dissimilar to that of polywater since, in contrast to the water cylinders and lamellae, the polywater exhibits a downfield shift compared with that of normal water in the NMR spectra."

From electrical and birefringence data we suggest that the two clear isotropic regions represent, respectively, a water-in-oil and an oil-in-water type of microemulsion. It is expected that as the amount of water increases, the lamellar structure will break down, and the water will form a continuous phase containing microdroplets of oil stabilized by potassium oleate and hexanol. The increase in electrical resistance above a ratio of water to oil of 1.0 is due mainly to the disruption of the lamellar structures, and the subsequent decrease above a ratio of 1.3 can be attributed to the formation of a continuous water phase (clear isotropic region). Therefore, the transition from water spheres to water cylinders to water lamellae to a continuous water phase represents the mechanism of phase inversion in microemulsions. FIGURE 4 schematically illustrates the mechanism of phase-inversion of microemulsions. It should be

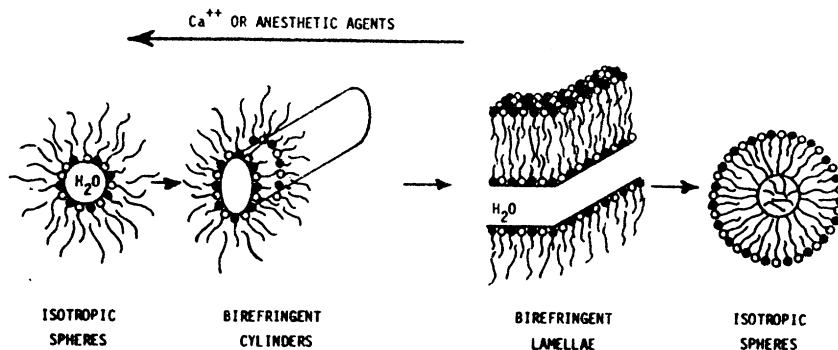


FIGURE 4. A schematic presentation of the mechanism of phase-inversion of microemulsions based on electrical, birefringence, and NMR measurements. The upper arrow shows the direction of changes induced by the addition of  $\text{Ca}^{++}$  or anesthetics.

emphasized that the formation of such structures, however, depends upon the concentration of emulsifiers, which in turn determines the area available per surface active molecule at the oil-water interface.<sup>10</sup> Recently Ohki and Aono<sup>11</sup> have reported theoretical conditions necessary for the formation of such structures in lipid-water systems. Phase inversion may result in a macro or a micro system, and the birefringent region may be transparent or turbid, with the degree of turbidity dependent upon the chemical constitution of the system. The transition from lipid spheres to lipid cylinders to lipid lamellae is known for soaps.<sup>12</sup> In this report I describe such transition for water.

FIGURE 5 shows the effect of  $\text{Ca}^{++}$  or anesthetics on the electrical resistance of the birefringent liquid-crystalline phase at the oil/water ratio 1.4. One would expect a decrease in the electrical resistance since  $\text{Ca}^{++}$  and anesthetics are ions and ionized molecules, respectively. Both  $\text{Ca}^{++}$  and anesthetics, however, increase the electrical resistance of the liquid-crystalline lamellar structure. Furthermore, it was observed that when the resistance reaches a maximal value, the birefringence completely disappears. The loss of birefringence suggests that the anesthetics or  $\text{Ca}^{++}$  destroy lamellar structure by altering the orientation of lipid molecules; the increase in the resistance suggests that the resulting

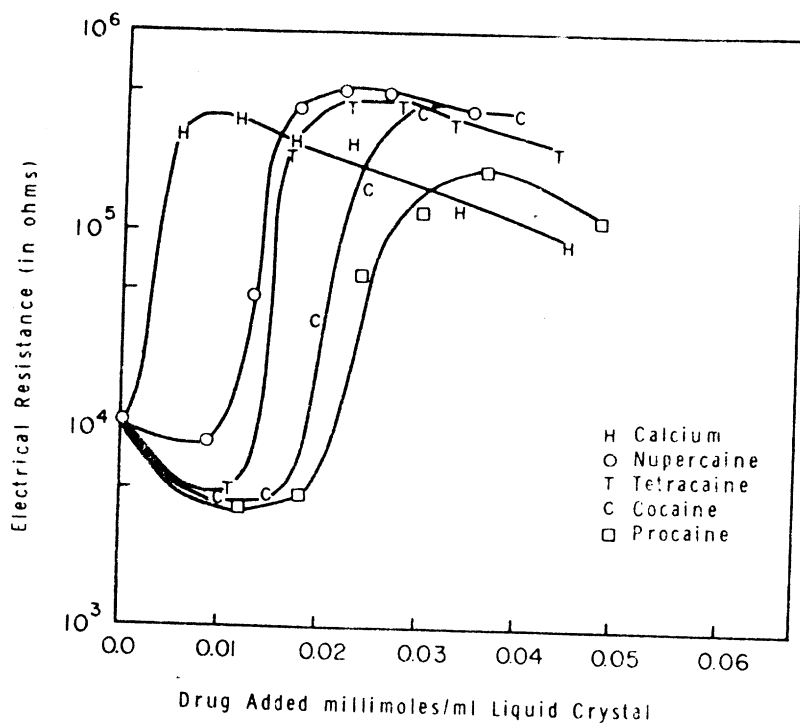


FIGURE 5. The effect of addition of  $\text{Ca}^{++}$  or anesthetic agents on the electrical resistance of the lamellar liquid-crystalline structures made of hexadecane, water, potassium oleate, and hexanol.

structure is an oil-continuous microemulsion (see FIGURE 4, upper arrow). The effectiveness of the anesthetics and  $\text{Ca}^{++}$  in equimolar amounts in increasing the electrical resistance was in the following order:  $\text{Ca}^{++} > \text{nupercaine} > \text{tetracaine} > \text{cocaine} > \text{procaine}$ . Significantly, this is the order of clinical potency for these anesthetics.

TABLE I shows the molecular ratio of drug to potassium oleate for the

TABLE I  
THE EFFECT OF ADDITION OF  $\text{CaCl}_2$  OR LOCAL ANESTHETICS  
ON ELECTRICAL RESISTANCE OF LAMELLAR LIQUID CRYSTALLINE STRUCTURES

Drug	Dose at 50% Increase in Electrical Resistance (mM Drugs/ml Liquid Crystalline phase)	Molar Ratio of Drug to Potassium Oleate *
$\text{Ca}^{++}$	0.00430	1.86/100
Nupercaine	0.01325	5.74/100
Tetracaine	0.01457	6.31/100
Cocaine	0.02020	8.75/100
Procaine	0.02355	10.2/100

\* Potassium oleate concentration: 2.2308 mM/ml liquid crystalline phase.

amount necessary to cause 50% of the maximum change in electrical resistance. If one assumes that all potassium oleate is at the interface in liquid-crystalline phase, then it is evident that five to ten drug molecules are able to influence 100 potassium oleate molecules in the liquid-crystalline structures. This is a striking example of long-range interaction between molecules at interfaces. It is known<sup>13</sup> that the concentration of various anesthetics in the brain is about 0.04 to 0.08 moles per liter of brain lipids when anesthesia occurs. Taking the average molecular weight of 800 for brain lipids, the molar ratio between the anesthetics and the brain lipids was found to be close to 0.04-0.08. Thus, liquid crystalline structures show almost the same sensitivity as brain lipids toward the anesthetics.

In summary, the electrical and birefringence measurements suggest that these anesthetics or  $\text{Ca}^{++}$  alter the interfacial forces and the orientation of lipid molecules, resulting in the destruction of the liquid crystalline structures. The effectiveness of anesthetics in causing these changes parallels their clinical potency. Also, 5-10 anesthetic molecules are able to influence 100 surfactant molecules in the liquid-crystalline structure.

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#### DISCUSSION

DR. BENDER (*Fairleigh Dickinson University, Rutherford, New Jersey*): When studying the change of resistance with the addition of a drug, you had



calcium ion present. Do you think that the activity of the drug is related to this divalent ion?

DR. SHAH: We used calcium ion because of the suggestions in the literature that this drug competes with calcium for binding on nerve membranes. We were studying the drug and calcium ion in a competitive sense.

DR. BENDER: The divalent ion is known to reverse emulsions. Do drugs have a similar effect?

DR. SHAH: Yes, but you must use higher concentrations of drugs.

DR. H. BERENDSEN: Dr. Shah, you did not explain the unusual chemical shift for water protons in lamellar systems.

DR. SHAH: Yes, it was unexpected; but it could simply reflect the arrangement of water molecules in 20–25 Å cylinders.

DR. M. U. PALMA: What were the frequencies at which the conductivity measurements were made?

DR. SHAH: We have done them at 60, 1,000 and 3,000 cycles/sec, and we find no frequency dependence.

DR. BERENDSEN: May I offer an explanation. We have worked on similar systems (potassium oleate in oriented systems), and we find that there is an angular-dependent shift which is due to a difference in the diamagnetic susceptibility of the lipid layer and the water layer. There is no necessity to invoke different properties for water in the new phase.

DR. LANGER (*University of California at Los Angeles, Los Angeles, California*): Did you test the effect of other ions on the resistance of micro-emulsions?

DR. SHAH: No, only calcium was studied.