Structural Investigation of Water/Lecithin/Cyclohexane Microemulsions by FT-IR Spectroscopy

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FT-IR spectra of water/lecithin/deuterated cyclohexane microemulsions as a function of water/lecithin molar ratio R (R = [water]/[lecithin]) at various volume fractions ($\emptyset$) of the micellar phase have been recorded at 25°C. After elimination of the small spectral contributions due to deuterated cyclohexane and normalization, the shape of the C–H stretching band due to lecithin has been found dependent upon R and $\emptyset$ whereas that of the O–H stretching band has been found dependent only upon R. The change in shape of the C–H band was interpreted in terms of a modification of the lecithin alkyl chain packing order. The analysis of the O–H band provides evidence that the hydroxylic groups of lecithin form strong hydrogen bonds whereas the added water is confined in bulk-like water domains.   © 1995 Academic Press, Inc.

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INTRODUCTION

Water can be solubilized in lecithin/hydrocarbon solutions forming thermodynamically stable monophasic systems (w/o microemulsions). An interesting peculiarity of these microemulsions is that a dramatic increase of viscosity (up to 10^6 cP) can be induced in these systems by changing some external parameters, i.e., the volume fraction of water plus lecithin ($\emptyset$), the molar ratio R (R = [water]/[lecithin]), or temperature. This behavior has been explained in terms of the formation of an entangled transient network of very long tube-like water/lecithin micelles (1).

Several investigations have been performed in order to clarify structural and dynamical properties of these molecular aggregates and their dimensional evolution with $\emptyset$, R, and temperature. In particular, it has been evidenced (2, 3) that at very low $\emptyset$ ($\emptyset$ = 0.0036) water addition induces a structural change from a dispersion of small spherical reversed micelles to very long and flexible cylindrical micelles which reach a hydrodynamic radius of 320 Å and a gyration radius of 615 Å at R = 8. In these conditions, an estimate (3) of the contour length (L) and of the cross-sectional radius (r) of water/lecithin micelles gave L = 4500 Å and r = 25 Å.

It has been also observed that their mean length increases with $\emptyset$ and decreases with temperature. Above a crossover $\emptyset$* or $1/T$* value, when the mean length reaches a threshold value, an extended dynamic network of entangled micelles is established and macroscopically the system appears in the gel state (4–7).

Since a change of shape and/or dimension of water/lecithin micelles should involve a modification of the alkyl chain packing order of lecithin molecules located at the micellar palisade layer and of the fraction of water molecules located near the water/lecithin interface, a measurable property sensitive to these features could be used, in principle, to probe the occurrence of micellar structural changes (8, 9).

With the aim to investigate the dimensional evolution of water/lecithin micelles with $\emptyset$ and R, we have selected the IR-spectroscopy technique as a suitable tool. It has been shown (10), in fact, that the lipid chain lateral packing, or intermolecular order, in biomembrane systems can be probed by observing the intensity changes occurring in the C–H stretching region (2800–3000 cm^{-1}). It has been also shown that the O–H stretching band (2800–3800 cm^{-1}) of water near interfaces is generally strongly different from that of bulk water (8, 9).

In this report we present an analysis of the FT-IR spectra of the water/lecithin/cyclohexane microemulsions as a function of R (R = 0, 1, 2, 3, 6, and 9) at various volume fractions ($\emptyset$) of water plus lecithin ($\emptyset$ = 0.01, 0.05, 0.1). As solvent, we have used deuterated cyclohexane in order to avoid that the giant C–H contribution of the undeuterated cyclohexane overwhelms the C–H and O–H bands of lecithin and the O–H band of water.

MATERIALS AND METHODS

Purification and purity test of soybean lecithin (L-α-phosphatidylcholine, >97%) have been described elsewhere (4, 5). After purification, lecithin was stored under vacuum at low temperature (<0°C) before use. The water content of the purified lecithin, determined by Karl Fisher titration, was 0.2 mol of water for 1 mol of lecithin. The structure of lecithin (11, 12) is reported in Fig. 1. Deuterated cyclohex-

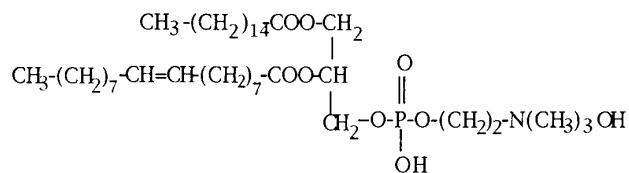[1] To whom correspondence should be addressed.

$$CH_3-(CH_2)_{14}COO-CH_2$$
$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COO-CH \quad O$$
$$CH_2-O-P-O-(CH_2)_2-N(CH_3)_3 \, OH$$
$$OH$$

FIG. 1. Schematic representation of the structure of L-α-phosphatidyl-choline.

ane (Cambridge Isotopes Laboratories, 99.7%) was used as received. Water was deionized and bidistilled.

The microemulsions were prepared by weight by first dissolving the lecithin in the cyclohexane and then adding the appropriate amount of water. Weight fractions of water plus lecithin were converted to volume fractions using the density values previously reported (4). By simple visual inspection, it was stated if each sample was a gel-like or a sol-like micellar solution at 25°C. The results of these observations are shown in Fig. 2.

FT-IR spectra of all the samples were recorded in the frequency range 1000–5000 cm^{-1} with a Bruker (IFS25) FT-IR spectrometer through a cell equipped with CaF$_2$ windows. The measurements were carried out at 25°C. The path length of the cell was choosen according to the volume fraction of water plus lecithin of the sample. Each sample was given 100 scans at a spectral resolution of 2 cm^{-1} before recording.

RESULTS AND DISCUSSION

A typical spectrum of water/lecithin/deuterated cyclohexane ($R = 3$, $\varnothing = 0.1$) in the frequency range 2500–4500 cm^{-1} is shown in Fig. 3. For comparison, the spectrum of pure deuterated cyclohexane is also reported. As can be seen, in the frequency range 2500–4500 cm^{-1} only small contributions are given by the deuterated cyclohexane. These contributions were removed by subtracting the spectrum of
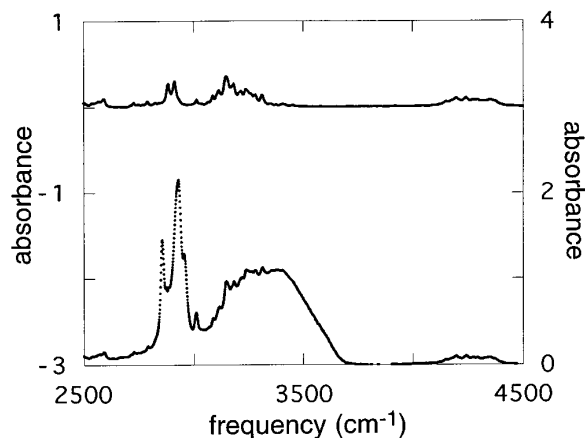

FIG. 3. Representative FT-IR spectrum (lower spectrum, right scale) of a water/lecithin/deuterated cyclohexane microemulsion ($R = 3$, $\varnothing = 0.1$). The upper line (left scale) is the spectrum of pure deuterated cyclohexane.

deuterated cyclohexane from that of the microemulsions. Moreover, all the spectra were reduced to the same height of the maximum of the O–H band. As examples, Figs. 4 and 5 show the normalized spectra of samples at $R = 0$ and $\varnothing = 0.05$ or $\varnothing = 0.1$, and at $R = 3$ and $\varnothing = 0.05$ or $\varnothing = 0.1$, respectively. For comparison, the spectrum of pure water is also reported.

An inspection of all the normalized spectra evidences that: (i) the band due to the C–H stretching modes of lecithin is composed mainly of a peak at 2855 cm^{-1}, a peak at 2930 cm^{-1} with a shoulder at 2956 cm^{-1}, and a small peak at 3010 cm^{-1}; (ii) the intensity of the peak at 2930 cm^{-1} is particularly sensitive to R and $\varnothing$ changes; (iii) the shape of the O–H band at $R = 0$ differs from that of pure water; and (iv) the shape of the O–H band depends upon R but is not significantly influenced by $\varnothing$.

In order to rationalize the observed changes of the peak intensity at 2930 cm^{-1}, it must be pointed out that the band around 2855 cm^{-1} is due to the methylene C–H symmetric stretching whereas the band around 2930 cm^{-1} is due to the methylene C–H antisymmetric stretching (11, 13–15). Moreover, according to Edwards *et al.* (15), the band at 2930 cm^{-1} is particularly sensitive to variation of intermolecular chain–chain interactions and can be taken as a probe of lateral packing order of the lecithin alkyl chains located in the micellar palisade layer. In particular, it has been suggested that a decrease of the A_{2930}/A_{2855} parameter, i.e., the ratio of the band intensity at 2930 cm^{-1} and 2855 cm^{-1}, indicates an increase in the lateral packing order of lecithin alkyl chain.

Figure 6 shows the ratio A_{2930}/A_{2855} as a function of $\varnothing$ at various R values. As can be seen, no correlation between A_{2930}/A_{2855} and the formation of gel-like micellar solutions is found (see also Fig. 2). Taking into account that an extensive entanglement of long cylindrical micelles should in-
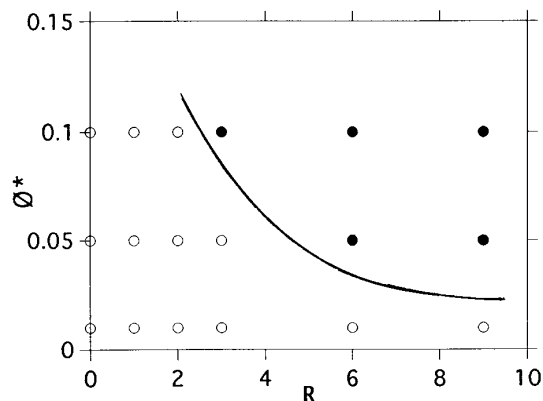

FIG. 2. Threshold values ($\varnothing^*$) of the lecithin volume fraction of the transition from sol-like to gel-like micellar solution vs R ($\bigcirc$) samples in the sol-like state, ($\bullet$) samples in the gel-like state.
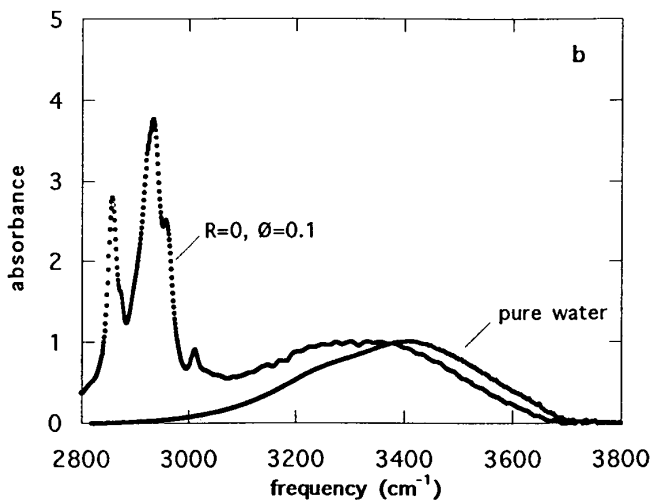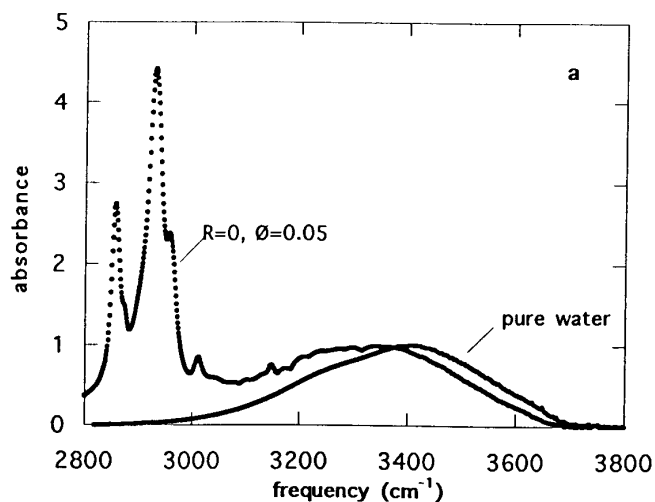
FIG. 4. Normalized C–H and O–H bands of water/lecithin/deuterated cyclohexane microemulsions in the frequency range 2800–3800 cm^{-1} at R = 0 and $\varnothing$ = 0.05 (a) or $\varnothing$ = 0.1 (b).

FIG. 5. Normalized C–H and O–H bands of water/lecithin/deuterated cyclohexane microemulsions in the frequency range 2800–3800 cm^{-1} at R = 3 and $\varnothing$ = 0.05 (a) or $\varnothing$ = 0.1 (b).

volve a change in the lateral packing order of the alkyl chain of lecithin and consequently in the A_{2930}/A_{2855} parameter, our finding can be taken as an indication that only a small fraction of lecithin molecules are involved at the entanglement sites of the micellar network. It is interesting to note that a similar result has been found by Capitani *et al.* (16) who did not observe in the ^{13}C NMR linewidth evidence of alkyl chain entanglement.

It can be also observed in Fig. 6 that, at each R, the ratio A_{2930}/A_{2855} remains nearly constant between $\varnothing$ = 0.01 and $\varnothing$ = 0.05 whereas it decreases significantly between $\varnothing$ = 0.05 and $\varnothing$ = 0.1. This behavior can be correlated to the dimensional evolution of water/lecithin micelles with $\varnothing$ (7). In fact, assuming that longer aggregates are characterized by slower conformational dynamics of lecithin alkyl chains, a tighter packing of lecithin molecules in the micellar palisade layer, i.e., a greater lateral packing order of lecithin
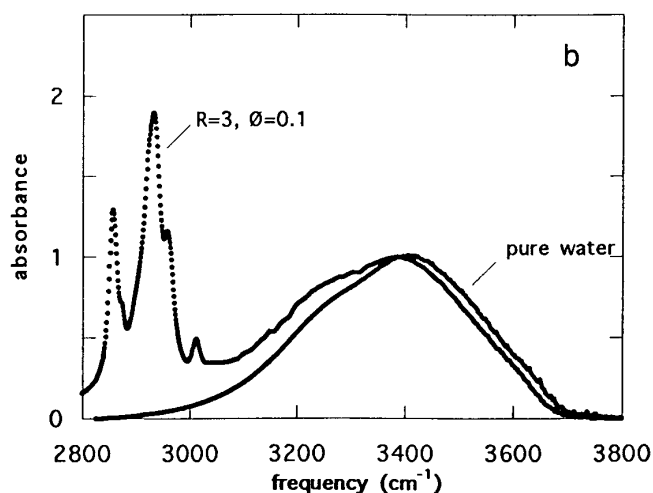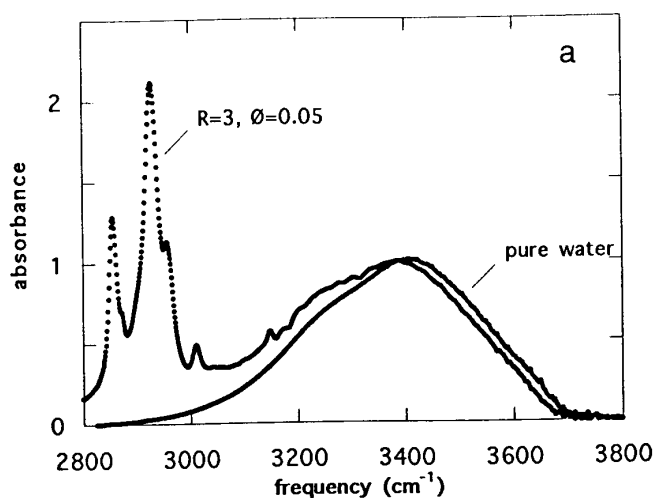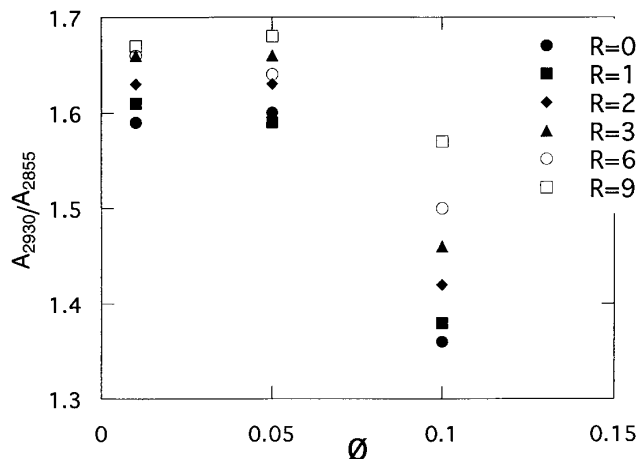

FIG. 6. A_{2930}/A_{2855} ratio (see text) as function of $\varnothing$ at various R values.
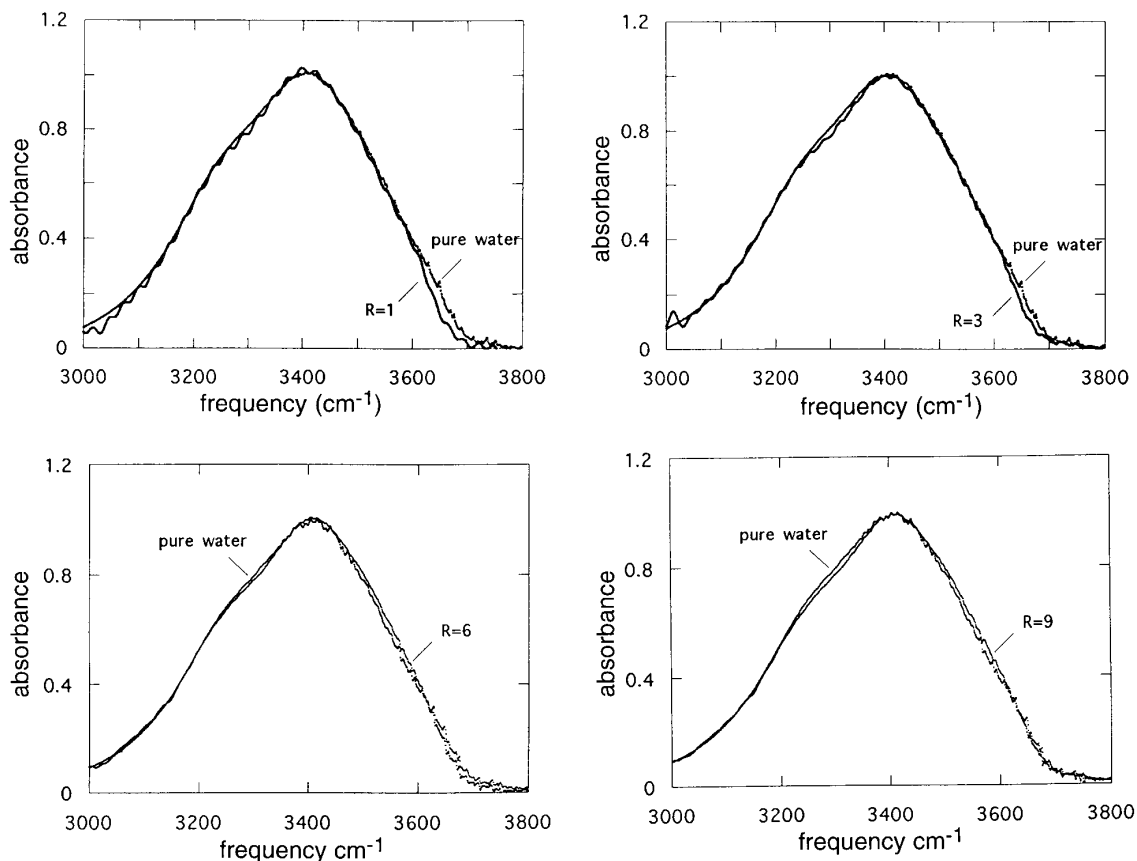
FIG. 7. Some representative normalized O–H bands of water in water/lecithin/cyclohexane microemulsions at various R and at $\emptyset = 0.1$.

alkyl chains, could be expected. Besides, Fig. 6 shows that, at each $\emptyset$ value, A_{2930}/A_{2855} increases with R. Evidently, a progressive increase of the water content determines some structural rearrangement of the water/lecithin interface involving a change in the lateral packing order of lecithin chains.

Concerning the O–H band, it must be observed that a contribution comes from the hydroxylic groups of the lecithin molecule (see Fig. 1). Besides, since the O–H band at $R = 0$ is shifted at lower frequencies than observed for bulk water, it can be argued that the hydrogen bond between lecithin molecules is stronger than that between water molecules.

In order to analyze the contribution of water to the O–H band, we have removed that due to lecithin by subtracting from the spectra at $R > 0$ the spectrum at $R = 0$. Some representative subtracted spectra, reduced to the same height of the maximum of the O–H band, are displayed in Fig. 7. As can be seen, surprisingly, we observe no significant difference with the spectrum of pure water. This finding is in contrast to the general opinion that interfacial water is less hydrogen bonded than pure water as a consequence of the strong interaction with the ionic head groups of surfactant molecules (8, 14). In particular, using the FT-IR technique,

it has been found by Maitra *et al.* (17) that water bound to lecithin micelles shows an O–H band centered at a frequency significantly lower than that displayed by bulk water. The use of a substantially different lecithin (lecithin extracted by egg yolk) could be invoked to justify the discrepancy between our result and that of Maitra *et al.* On the other hand, it is interesting to note that a result consistent with our finding was already observed in a 10% by weight suspension of dipalmitoylphosphatidylcholine unilamellar vesicles with an approximate radius of 100–500 Å in water (18). Moreover, a Monte Carlo study (19) has shown that the influence of phosphatidylcholine head group interface on the polarization of the closest water layers is nearly absent when the lecithin dipoles are antiparallel.

Further investigations are being planned in our laboratory in order to better clarify the state of water within lecithin reversed micelles and the intermicellar interactions responsible for the transition from a sol-like to a gel-like micellar solution.

ACKNOWLEDGMENTS

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