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Water Diffusion in Polymer-like Reverse Micelles. 2. Composition Dependence[†]

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A pulsed field gradient FT ^{1}H NMR study on the system soybean lecithin/water/perdeuterated cyclohexane, covering a wide range of compositions, is presented. The water diffusion was found to be Gaussian with a well-defined, time-independent, single diffusion coefficient. After the contribution from the diffusion of water dissolved in the cyclohexane has been properly taken into account, all the data follow the same master curve as a function of the water to lecithin molar ratio (W_0). The dominating mechanism for the water diffusion was found to be the motion inside giant wormlike reverse aggregates mediated by an interaggregates exchange with a characteristic time on the order of microseconds. For all the lecithin concentrations investigated, increasing the value of W_0 increases the water diffusion up to $W_0 = 15$, where a structural transition toward spherical aggregates causes a decrease in the water diffusion.

1. Introduction

Many studies concerning the microstructure in the W/O microemulsion (L_2 phase) formed in the lecithin/water/cyclohexane system have been carried out using mainly light scattering (LS),[1–4] small-angle neutron scattering (SANS),[2,5] and NMR techniques.[6–9] In contrast to the well-known L_2 systems with Na(AOT), where reverse micelles can swell to large water contents, retaining a spherical shape,[10] the microstructure in the L_2 phases with lecithin is strongly dependent on the solubilized water to surfactant molar ratio, W_0, and the formation of gellike viscoelastic solutions can be observed in several nonpolar solvents.[11] Simple spherical structures are not a general feature of surfactant-based systems in nonaqueous media. Indeed, polymer-like W/O microemulsion aggregates have been reported also with copper(II) dialkyl carboxylates,[12] di-

dodecyldimethylammonium bromide (DDAB),[13,14] and various metal and quaternary ammonium AOT surfactants.[15]

Generally, cylindrical reverse aggregates are found at low W_0 values, while at higher W_0 a shape transition is encountered, where the aggregates become spherical. In the case of several DDAB and PFPE W/O microemulsions for example the experimental conductivity and diffusion data, interpreted in term of the DOC model, pointed out a gradual evolution of the microstructure from bicontinuity (at low water content) to disconnected W/O droplets (at high water content).[14,16]

In colloid science, the polymer-like or wormlike micelles have attracted a considerable amount of attention because their behavior is expected to be reminiscent of "equilibrium polymers" (see next sections). Moreover, the technological use of wormlike micelles is related to their exceptional viscoelastic properties. As such, the lecithin L_2 system is particularly interesting since it shows polymer-like properties, the properties of which can be tuned with the parameter W_0. Further advantages in investigating wormlike reverse micelles is the possibility to study the bare micellar properties with little ambiguity since organic solvents, unlike water, do not induce complicating effects originating from electrostatic interactions. Finally, phospholipid-based W/O microemulsions can be successfully used as membrane model to study the behavior of large guest molecules such as enzymes[17] and photochemically active proteins.[18]

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† This paper is dedicated to the memory of Prof. Americo Inglese (1946–1998).

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(1) Schurtenberger, P.; Magid, L. J.; King, S. M.; Lindner, P. *J. Phys. Chem.* **1991**, *95*, 4173.
(2) Schurtenberger, P.; Magid, L. J.; Lindner, P.; Luisi, P. L. *Prog. Colloid Polym. Sci.* **1992**, *89*, 274.
(3) Schurtenberger, P.; Cavaco, C. *J. Phys. 2* **1993**, *3*, 1279. Schurtenberger, P.; Cavaco, C. *J. Phys. 2* **1994**, *4*, 305. Schurtenberger, P.; Cavaco, C. *J. Phys. Chem.* **1994**, *98*, 5481.
(4) Schurtenberger, P.; Cavaco, C. *Langmuir* **1994**, *10*, 100.
(5) Schurtenberger, P.; Jerke, G.; Cavaco, C.; Pedersen, J. S. *Langmuir* **1996**, *12*, 2433.
(6) Capitani, D.; Rossi, E.; Segre, A. L.; Giustini, M.; Luisi, P. L. *Langmuir* **1993**, *9*, 685. Capitani, D.; Segre, A. L.; Dreher, F.; Walde, P.; Luisi, P. L. *J. Phys. Chem.* **1996**, *100*, 15211.
(7) Angelico, R.; Colafemmina, G.; Della Monica, M.; Palazzo, G.; Giustini, M.; Ceglie, A. *Prog. Colloid Polym. Sci.* **1997**, *105*, 1184.
(8) Angelico, R.; Cirkel, P. A.; Colafemmina, G.; Palazzo, G.; Giustini, M.; Ceglie, A. *J. Phys. Chem. B* **1998**, *102*, 2883.
(9) Angelico, R.; Olsson, U.; Palazzo, G.; Ceglie, A. *Phys. Rev. Lett.* **1998**, *81*, 2823.
(10) Eastoe, J.; Robinson, B. H.; Thorn-Leeson, D. *Adv. Colloid Interface Sci.* **1991**, *35*, 1 and references therein.
(11) Scartazzini, R.; Luisi, P. L. *J. Phys. Chem.* **1989**, *92*, 829.
(12) Terech, P.; Schafhauser, V.; Maldivi, P.; Geunet, J. M. *Europhys. Lett.* **1992**, *17*, 515.
(13) Fontell, K.; Ceglie, A.; Lindman, B.; Ninham, B. W. *Acta Chem. Scand., Ser. A* **1986**, *40*, 247. Eastoe, J.; Heenan, R. K. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 487. Knackstedt, M. A.; Ninham, B. W. *Phys. Rev. E* **1994**, *50*, 2839. Knackstedt, M. A.; Monduzzi, M.; Ninham, B. W. *Phys. Rev. Lett.* **1995**, *75*, 653.
(14) Hyde, S. T. *J. Phys. Chem.* **1989**, *93*, 1458. Hyde, S. T.; Ninham, B. W.; Zemb, T. *J. Phys. Chem.* **1989**, *93*, 1464.
(15) Petit, C.; Lixon, P.; Pileni, M. P. *Langmuir* **1991**, *7*, 2620. Eastoe, J.; Robinson, B. H.; Heenan, R. K. *Langmuir* **1993**, *9*, 2820. Caboi, F.; Capuzzi, G.; Baglioni, P.; Monduzzi, *J. Phys. Chem.* **1997**, *101*, 10205.
(16) Monduzzi, M.; Knacksted, M. A.; Ninham, B. W. *J. Phys. Chem.* **1995**, *99*, 17772.
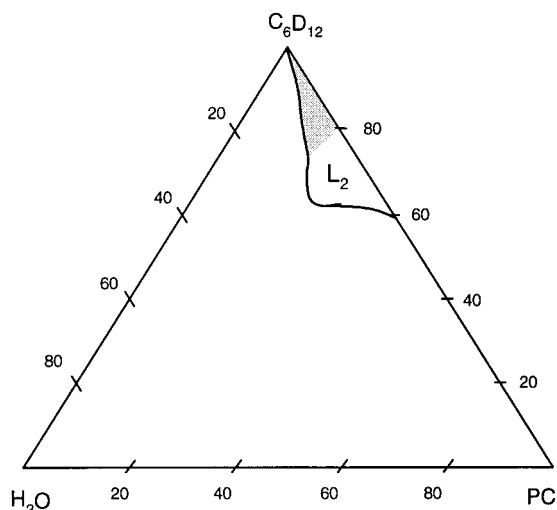(17) Peng, Q.; Luisi, P. L. *Eur. J. Biochem.* **1990**, *188*, 471.

Figure 1. Phase diagram (wt %) of the system $H_2O/PC/C_6D_{12}$. (Only the extension of the L_2 phase is shown.) The shaded area indicates the range of compositions investigated in the present study.

A recent study based on lecithin NMR self-diffusion measurement,[9] is in agreement with the idea (previously suggested on the basis of LS data[1]) that the L_2 gellike phase is made of giant wormlike micelles forming a transient network similar to that found in semidilute polymer solutions. The contour length L of these wormlike micelles increases upon increasing the value of W_0 up to $W_0 = 10-12$ in cyclohexane. We have recently shown that, for lecithin concentration of 0.2 mol kg^{-1}, further water addition induces a rod-to-sphere transition, and at higher water contents the structure approaches spherical reverse micelles, coexisting with excess water at the phase boundary around $W_0 = 26-28$.[8] The viscosity of samples follows the same trend when measured as function of the water content: At low W_0 it increases significantly in agreement with the water-induced micellar growth, and above $W_0 \approx 10$ it falls again. The whole W_0 dependence of the viscosity can be described as bell-shaped.[2]

In this report we focus on water dynamics in the L_2 region and we present experimental water self-diffusion data at 25 °C from several water and oil dilution lines inside the shaded area of the L_2 phase shown in Figure 1. Subsequently, we briefly discuss possible diffusion processes for water molecules and conclude, on the basis of the present data and previously published lecithin diffusion data,[9] that the dominating water diffusion process is diffusion within the wormlike micelles with rapid exchange between aggregates.

2. Materials and Methods

Materials. Soybean lecithin (Epikuron 200) was a generous gift from Lucas Meyer AG and was used without further purification. To minimize the ^1H NMR signal from the solvent protons we used perdeuterated cyclohexane purchased from Dr. Glaser and Aldrich. Water was Millipore filtered. Samples were prepared as previously described.[7]

Methods. Self-diffusion coefficients measurements were carried out by pulsed gradient spin–echo techniques on three different NMR spectrometers: BS-587A NMR (Tesla) operating

at 80 MHz for ^1H, experimental setup as reported elsewhere;[19] JEOL FX60 NMR operating at 60 MHz for ^1H; Bruker DMX 200 operating at 200 MHz for ^1H.

The basic Fourier transform-pulsed gradient spin–echo (FT-PGSE) NMR experiment generates a spin–echo by a 90°–180° pulse sequence with a magnetic field gradient G applied during a time δ in the form of two pulses separated by a time Δ (from leading edge to leading edge), one pulse before and one after the 180° pulse.[20] Fourier transformation of the digitized second half of the echo separates the contributions to the echo from the different signals in the spectra.

The amplitude A of a given NMR resonance is

$$A = A_0 \exp[-\gamma^2 G^2 \delta^2 D(\Delta - \delta/3)]$$

where A_0 is the echo amplitude in absence of gradient, γ is the gyromagnetic ratio, and D is the self-diffusion coefficient of the molecule. Experiments performed on both BS-587A (80 MHz) and JEOL FX-60 (60 MHz) were carried out at constant Δ and G values for a series of δ values in the interval between 2 and 50 ms. The above equation were then fitted with a nonlinear least-squares fitting program to the measured $A(\delta)$ values. Prior to each experimental series the factor $(\gamma G)^2$ was obtained by performing measurements on samples with known self-diffusion coefficients.[21] On the Bruker DMX-200 a FT-PGSE sequence with constant Δ and δ values for a series of G in the interval between 0.1 and 2 T m^{-1} with a phase cycling of 4 steps was employed. In some case, especially for samples at low lecithin concentration and low W_0, the echo attenuation of the water signal was detected using a stimulated echo pulse sequence[22] performed on the same instrument by varying the gradient strength G. Explicit care was undertaken to confirm experimentally the unrestricted three-dimensional property of the water diffusion measured under the present conditions. This was done by varying the observation time, and thereby the observed mean-square displacement of water molecules, by varying the time Δ in the pulse sequences above-described for most of the samples. The accuracy in the measured diffusion coefficients was better than ±5% as determined by repeated measurements. All experiments were carried out at 25 ± 0.5 °C as measured by a calibrated copper–constantan thermocouple.

3. Results

The data presented here were obtained using three different NMR spectrometers working at different fields. Furthermore, the experiments were performed in two different laboratories on samples prepared with two different stocks of soybean lecithin (PC). In this section we will illustrate the reproducibility of the data obtained, the time scale dependence of the echo decays, and the influence of both lecithin concentration (C_{PC}) and W_0 (molar ratio H_2O/PC) on the diffusional behavior of the water.

3.1. Reproducibility. Under all the experimental conditions (vide infra) the echo decay is accounted with a single-exponential decay (when plotted vs the relevant experimental parameters; see above) giving a value of the self-diffusion coefficient, D_{obs}. Experiments carried out immediately after the samples preparation usually give D_{obs} values that are significantly different for samples sharing the same composition (this is more evident for samples at low C_{PC}). However, after an aging of 3–6 days the echo decay becomes characterized by a constant D_{obs} value. Such a value is independent from the pulse sequence used and from the experimental set up. More important, the agreement among results obtained from different samples sharing the same composition is quite good (the differences are below the uncertainty in each single

(18) Palazzo, G.; Giustini, M.; Mallardi, A.; Della Monica, M.; Ceglie, A. *Prog. Colloid Polym. Sci.* **1996**, *102*, 19. Agostiano, A.; Catucci, L.; Della Monica, M.; Mallardi, A.; Palazzo, G.; Venturoli, G. *Bioelectrochem. Bioenerg.* **1995**, *38*, 25. Mallardi, A.; Palazzo, G.; Venturoli, G. *J. Phys. Chem.* **1997**, *101*, 7850. Mallardi, A.; Giustini, M.; Palazzo, G. *J. Phys. Chem.* **1998**, *102*, 9168.

(19) Giustini, M.; Palazzo, G.; Colafemmina, G.; Della Monica, M.; Giomini, M.; Ceglie, A. *J. Phys. Chem.* **1996**, *100*, 3190.
(20) Tanner, J. E.; Steskjal, E. O. *J. Chem. Phys.* **1968**, *49*, 1768.
(21) Mills, R. *J. Phys. Chem.* **1973**, *77*, 685–688.
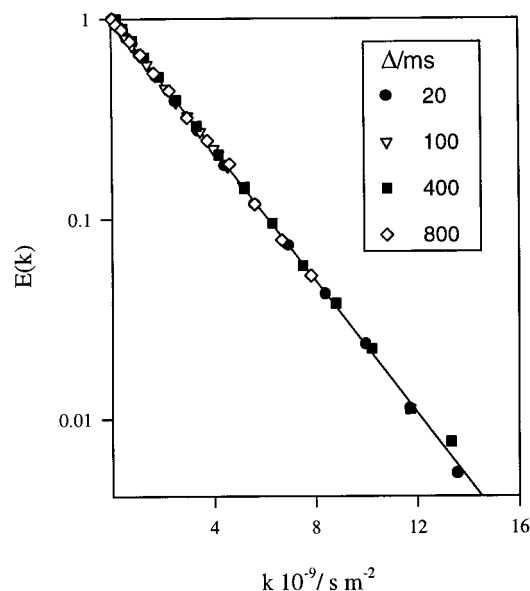(22) Stilbs, P. *Prog. NMR Spectrosc.* **1987**, *19*, 1–45.

Figure 2. Water echo attenuation $E(k) = A/A_0$, where $k = \gamma^2 G^2 \delta^2 (\Delta - \delta/3)$ of a sample at $W_0 = 4$ and $C_{PC} = 0.198$ mol kg^{-1}. The symbols indicate experiments performed at different values of Δ. The line denotes the best fit.

determination). This feature is conserved for long periods (up to 3 months for samples stored in the dark at constant temperature).

After longer aging times (1–3 months), some degradation of the samples occurs, with subsequent changes in the D_{obs}. In summary, samples that are equilibrated and still perfectly transparent give data which are reproducible (also when we compare results coming from different instruments, sequences, and samples).

3.2. Time Scale Dependence. The echo decay of the water peak for lecithin organogels in cyclohexane was recorded at different pulse interval times (Δ). For *all* the samples examined the decay was found to be monoexponential and time independent. A typical result is shown in Figure 2, where the normalized water echo attenuation at $W_0 = 4$ and $C_{pc} = 0.2$ mol/kg is reported for different Δ, from 20 to 800 ms. Since, the water echo decay is always monoexponential and time independent, the observed D_{obs} can be confidently used to characterize the diffusional behavior of water. This is different from the lecithin echo decays, which are nonexponential and strongly dependent on the value of Δ.[9]

3.3. Dependence on C_{PC} and W_0. The self-diffusion coefficients of water were collected along several water and oil dilution lines and are shown in Figure 3 in a three-dimensional plot. It is interesting to note the main features of the water diffusion behavior:

(i) At high C_{PC} (0.075 m and above) the D_{obs} values are strongly dependent on W_0, showing a bell-shaped plot when plotted vs W_0. In this range of concentrations, the dependence of D_{obs} on the lipid concentration is negligible. In particular, for $7 \leq W_0$ all the samples at a given W_0 share about the same value of D_{obs}, independent from the value of C_{PC}.

(ii) At low lecithin concentration (below 0.05 m) the influence of the ratio water/lecithin on D_{obs} is small. At these compositions, the water diffusion decreases in a monotonic way with the increase of C_{PC}. In the case of fast water exchange, the value of D_{obs} is a population-weighted average of the diffusion coefficients of water solubilized in the bulk oil ($D_{w,o}$) and of the water molecules confined
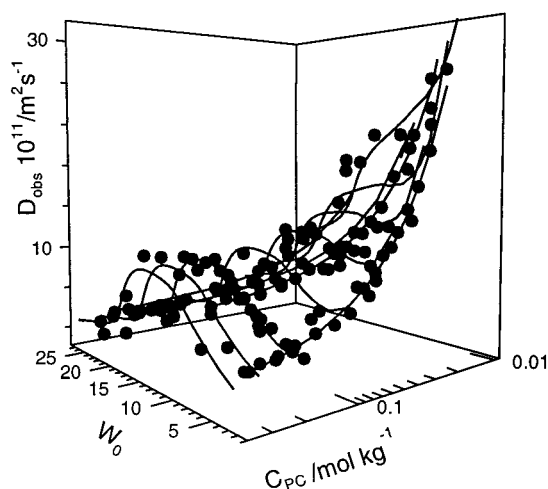

Figure 3. Experimental self-diffusion coefficients of water as a function of both W_0 and C_{PC}. The lines are guides for the eye.
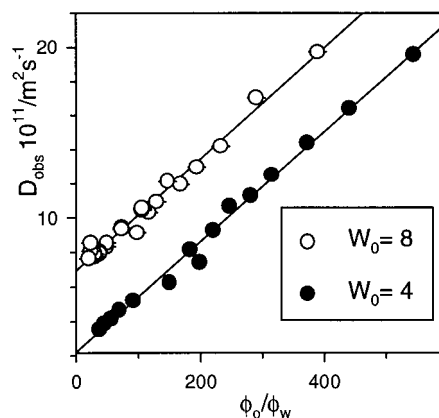

Figure 4. Observed water diffusion along oil dilution lines for two different W_0 values. The lines are the best fit to eq 2.

in the aqueous core of the aggregates ($D_{w,aq}$):[23]

$$D_{obs} = \frac{\phi_{w,o}}{\phi_w}(D_{w,o} - D_{w,aq}) + D_{w,aq} \tag{1}$$

Here, ϕ_w is the (overall) water volume fraction and $\phi_{w,o}$ is the volume fraction of the water solubilized in the continuous oil phase ($\phi_{w,o} \ll \phi_w$). Assuming $D_{w,o} \gg D_{w,aq}$, eq 1 can be written as

$$D_{obs} = \frac{\phi_o}{\phi_w}KD_{w,o} + D_{w,aq} \tag{2}$$

as long as the ratio $\phi_{w,o}/\phi_o = K$ is constant (i.e. the oil is saturated with water; ϕ_o denotes the oil volume fraction).

Equation 2 predicts a linear dependence of D_{obs} on the ratio between oil and water volume fraction. This was experimentally found as shown in Figure 4 for two different W_0 values. It can be noticed that the two straight lines of Figure 4 have the same slope $KD_{w,o} = (3.1 \pm 0.3) \times 10^{-13}$ m^2 s^{-1}, as expected since the samples share the same diffusion and solubility of water in the oil. The values reported for water solubility in cyclohexane range from[24] 1.3×10^{-4} to[25] 4.7×10^{-4} (in molar fraction units). These values correspond to $D_{w,o} = (15-3) \times 10^{-9}$ m^2 s^{-1},

(23) Skurtveit, R.; Olsson, U. *J. Chem. Phys.* **1992**, *96*, 8640.
(24) Scartazzini, R. Dissertation No. 9186, ETH Zürich, 1990.
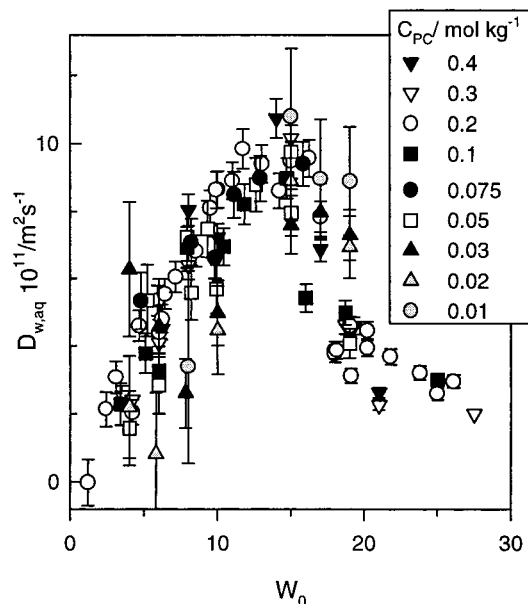(25) Black, C.; Joris, G. G.; Taylor, H. S. *J. Chem. Phys.* **1948**, *16*, 537.

Figure 5. Self-diffusion coefficients of water confined in the aqueous core of the aggregates, $D_{w,aq}$, as a function of W_0. The observed diffusion of Figure 3 was corrected according to eq 2 (see text). The PC concentrations are indicated by different symbols.

indicating that the water diffusion constant in cyclohexane is higher than in neat water, which is expected since in the oil the hydrogen bonding is absent. The values of the intercepts, on the other hand, strongly depend on the water-to-lecithin ratio. It is possible to subtract the contribution of free water from all the data and plot $D_{w,aq}$ = $D_{obs} - KD_{w,o}\phi_o/\phi_w$ for all the lecithin concentration as a function of W_0, thus transforming the data of Figure 3 into the more homogeneous set shown in Figure 5, where the uncertainties coming from the correction for the free water are shown as error bars. The uncertainty on the experimental data is around 5%; thus, the higher D_{obs}, the higher the associated error. Furthermore, the contribution to the error in $D_{w,aq}$ due to the term $(KD_{w,o})\phi_o/\phi_w$ increases with dilution. As a consequence, we have large errors associated with $D_{w,aq}$ at low PC concentration. Despite these large uncertainties, it is clear from Figure 5 that the corrected values $D_{w,aq}$ follow the same trend. Here the influence of the lecithin concentration has essentially disappeared and all the data obey the same W_0 dependence.

4. Discussion

We have seen that the water diffusion is time independent and Gaussian (exponential echo decay). This means that the water molecules although confined inside the aggregates have a choice of paths after a certain time, τ, which is much shorter than the observation time. In other words, on the experimental time scale we can describe the water motion as a random walk. Basically, in our system there are four diffusion mechanisms consistent with these features.

Aggregate Diffusion. If the water molecules are confined to reverse micelles they will diffuse with the aggregates and we expect $D_w \approx D_{Lec}$. However, in semidilute solutions of polymer-like reverse micelles the micellar motion should proceed through the reptation mechanism (for $\tau_{break} > \tau_{rep}$). In this case τ is the reptation

time (τ_{rep}) and the micellar self-diffusion coefficient is given by[26]

$$D_{rep} = KT(6\pi\eta\xi)^{-1} (L/g\lambda)^{-2}$$

where ξ denotes the correlation length and g is the number of characteristic step length (λ, which, for an isolated wormlike chain, can be identified with the persistence length) in a blob. For our systems we expect $D_{rep} \ll 10^{-12}$ m² s⁻¹; this is confirmed by the fact that the lecithin diffusion (which is slower than water diffusion) is unaffected by the micellar motion.[9]

All the other mechanisms involve a random choice among different curvilinear paths inside the tubular aggregates. The choices are as follows:

Breaking and Recombining of Micelles. In this case τ is related to the lifetime of a micelle, τ_{break}.[27] Lecithin reverse micellar solutions resemble in many ways polymer solutions, but there are also some particular differences. The micellar contour length is not constant but the result of a thermodynamic equilibrium, and generally the contour length increases with the concentration. Because they are self-assembled aggregates of individual molecules, they also possess a finite lifetime in contrast to classical polymer chains which are held together with covalent bonds. The micellar aggregates can break and recombine and are therefore often referred to as a "living polymer". In principle, the molecular motion of both water and lecithin are strongly affected by the micellar dynamics. We have recently shown that the PC diffusion behaves as expected for lateral diffusion along *unbreakable*, *unbranched* wormlike micelles.[9] This result was obtained in experiments performed in a wide range of Δ, up to 1.5 s, suggesting that micellar dynamics can be neglected in experiments performed on a subsecond time scale.

Presence of Branches. In this case τ is the time required for a water molecule to move from one branch point to another. The lecithin motion follows the prediction for disconnected wormlike reverse micelles at least for time scales up to 1.5 s.[9] The local curvilinear diffusion constant of lecithin in a bilayer is around 10^{-12} m² s⁻¹. This means that the curvilinear mean length explored in 1.5 s is about 2 μm. Water inside the reverse micelles should diffuse faster than PC. Measurements on PC/water lamellar phase indicate, indeed, a local self-diffusion coefficient of about 10^{-10} m² s⁻¹.[28] However, the water echo decay is still exponential in experiments performed with $\Delta = 20$ ms. The curvilinear diffusion length of water on this short time scale is comparable with the root-mean-square curvilinear displacement experienced by PC molecules for 1.5 s, and since the lecithin does not see any physical connection between micelles, the Gaussian behavior of water diffusion cannot be related to the presence of branches. This is supported from the phase diagram in which no bicontinuous mesophases are present.[29]

Exchange. As a whole, the above-reported discussion leaves exchange between micelles as a likely reason a Gaussian diffusion is observed for water in the present study. Indeed, the choice of paths can be achieved by migration of water from one giant wormlike micelle to another. It is evident from Figure 5 that the contribution to the water diffusion due to the water confined inside the

(26) de Gennes, P. G. In *Scaling Concepts in Polymer Physics*; Cornell University Press: New York, 1979.
(27) Schmitt, V.; Lequeux, F. *Langmuir* **1998**, *14*, 283 and references therein.
(28) Wassel, S. R. *Biophys. J.* **1996**, *71*, 2724.
(29) Angelico, R.; Olsson, U. To be published.

aggregates is strongly dependent on the W_0 and independent from the lecithin concentration. These features are fully consistent with the water exchange mechanism since for all the other diffusional processes abovementioned a dependence on the lecithin concentration is expected.[27] The $D_{w,aq}$ independence from the PC concentration gives further insight into the diffusional motion. For "living polymer" systems (in our case systems at a fixed W_0), a concentration dependence of the micellar contour length, L, is expected. Such a dependence should be of the form $L \propto C_{PC}^\alpha$, where mean-field models and scaling arguments predict $\alpha = 0.5$ and $\alpha = 0.6$, respectively.[30] Experimental studies have confirmed the power law dependence of L on C_{PC} indicating an anomalous growth of the micelles with PC concentration ($\alpha \approx 1$).[3] Keeping in mind this strong concentration dependence of the micellar contour length, it is clear that Figure 5 (where $D_{w,aq}$ depends only on W_0 for 0.01 mol kg^{-1} $\leq C_{PC} \leq$ 0.4 mol kg^{-1}) excludes any influence of the aggregate size on the water diffusion. This fact allow us to neglect mechanisms in which the steps of the random walk are related to the micellar size such as the mechanism proposed, for aqueous systems, by Kato et al.[31] and that governing the probe diffusion in the "waiting" regime of Morié et al.[32]

In Figure 5, $D_{w,aq}$ increases with W_0 up to 16 and decreases for higher values. We have, recently, rationalized (for high PC content) the drop in $D_{w,aq}$ in terms of a rod-to-sphere transition.[8] Such a transition is related to the problem of accommodating an amount of water greater than $(a_s r_c/2 v_w - v_h/v_w)$ molecules of water per lecithin molecule in cylindrical micelles with a fixed curvature.[8] Using the literature values for the molecular water volume (v_w), the lecithin polar head area (a_s) and volume (v_h), and the radius of the aqueous core (r_c) obtained by SANS, one find $W_0 \approx 15$ as the maximum water uptake compatible with a cylindrical geometry.[8] The calculations were made on the basis of a purely geometrical model, and thus, they hold for all the concentrations. Once the decrease in $D_{w,aq}$ for $W_0 > 16$ has been explained it remains to clarify the increase in water diffusion for W_0 values from 1 to 16.

The fast exchange of water revealed in PGSE-NMR experiments means that the residence time of water molecules (τ) is shorter than Δ. In the time τ, before migration either into another aggregate and into the bulk, the water travels a curvilinear distance $\Lambda = (2D_c\tau)^{1/2}$, where D_c is his local (curvilinear) self-diffusion coefficient. We can describe the micellar contour as freely joint elementary steps of length λ (right of Figure 6). Accordingly, Λ can be depicted as the portion of such a contour delimited by two adjacent points where the migration happens (dots in Figure 6). From the point of view of water diffusion, the *disconnected* reverse micellar system is equivalent to a tubular *branched* network (although *there are not* physical connection between micelles). In other words, the points where the water exchange takes place act as "virtual branch points" (Figure 6). Since the path of length Λ between two virtual branches can be, always, described as N freely joint elementary steps, we can identify two situations for water migration:

(i) $\lambda < (2D_c\tau)^{1/2}$; in this case the number of elementary steps before migration is given by the number of statistical lengths between two virtual branch points ($N = \Lambda/\lambda$). Therefore, the water diffusion can be described as diffusion in a network of interconnected *random coiled* pipes (Figure
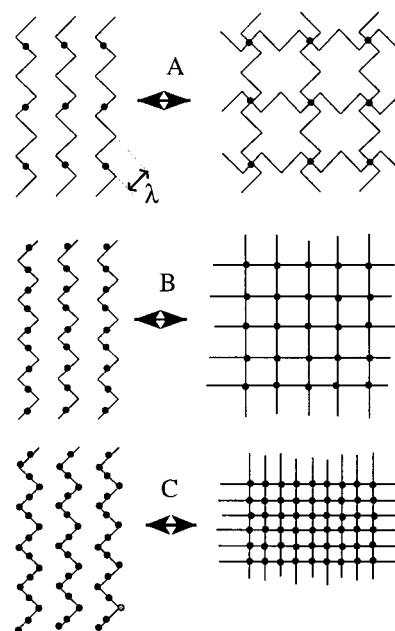

Figure 6. Schematic representation of the water diffusion behavior (see text). On the left side are represented the polymer-like micelles whose contour length is approximated as freely joint steps of length λ. The contour distance between two dots represents $(2D_c\tau)^{1/2}$. On the right side are represented the equivalent branched network: (A) $(2D_c\tau)^{1/2} = 3\lambda$; (B) $(2D_c\tau)^{1/2} = \lambda$; (C) $(2D_c\tau)^{1/2} = \lambda/2$. As long as $(2D_c\tau)^{1/2} \leq \lambda$, the equivalent branched network is always made of stiff connections whatever is the value of $(2D_c\tau)^{1/2}$ (i.e. $N = 1$), while for $(2D_c\tau)^{1/2} > \lambda$ it is formed by random coiled connections (e.g. $N = 3$ in (A)).

6A). Accordingly, $D_{w,aq} = D_c/3N$, which is always lower than the value expected for interconnected stiff tubes ($D_c/3$).

(ii) $\lambda \geq (2D_c\tau)^{1/2}$; the water molecules undergo a hopping motion before to travel along a length λ. Figure 6B schematically shows the case for $\lambda = (2D_c\tau)^{1/2}$, while $\lambda > (2D_c\tau)^{1/2}$ is illustrated in Figure 6C. Please note that in both the cases Λ is described by a *single* elementary step (the only difference is the length of such a step). In other words for $\lambda \geq (2D_c\tau)^{1/2}$, N equals always the unity and the water diffusion can be described as diffusion in a network of interconnected *stiff* pipes ($D_{w,aq} = D_c/3$). It should be stressed that case ii implies a dependence of $D_{w,aq}$ only on the local properties (D_c) while in case i a dependence on the statistical length is expected ($N = (2D_c\tau)^{1/2}\lambda^{-1}$).

It is possible to discriminate between these two cases if the values of D_c are known at different W_0. The mobility of water in the presence of phospholipid headgroups is strongly related to the hydration of the latter. A recently published study performed on PC/water oriented lamellar phases at different phospholipids hydration (W_0) revealed that the water diffusion parallel to the lamellar plane (D_c) increases with W_0, from 1.3×10^{-10} m^2 s^{-1} at $W_0 = 5$ to 4.1×10^{-10} m^2 s^{-1} at $W_0 = 19$.[28] In a first approximation we can use as D_c the values from a second-order polynomial interpolation of the data of ref 28. A straightforward way to discriminate between cases i and ii is to plot $D_{w,aq}/D_c$ and $D_{w,aq}/D_c^{1/2}$ as a function of W_0.

For $\lambda < (2D_c\tau)^{1/2}$, $D_{w,aq}/D_c^{1/2} \propto \lambda/\tau^{1/2}$, which is expected to be constant as long as τ and λ are W_0 and C_{PC} independent. On the other hand, $D_{w,aq}/D_c$ should scale as $D_c^{-1/2}$ in a plot against W_0.

For $\lambda \geq (2D_c\tau)^{1/2}$ we expect an opposite trend, a constant value for $D_{w,aq}/D_c = 1/3$ and $D_{w,aq}/D_c^{1/2} = D_c^{1/2}/3$.

In Figure 7A,B we plot, for all the PC concentrations,

(30) Cates, M. E.; Candau, S. J. *J. Phys.: Condens. Matter* **1990**, *2*, 6869 and references therein.

(31) Kato, T.; Terao, T.; Seimiya, T. *Langmuir* **1994**, *10*, 4474.

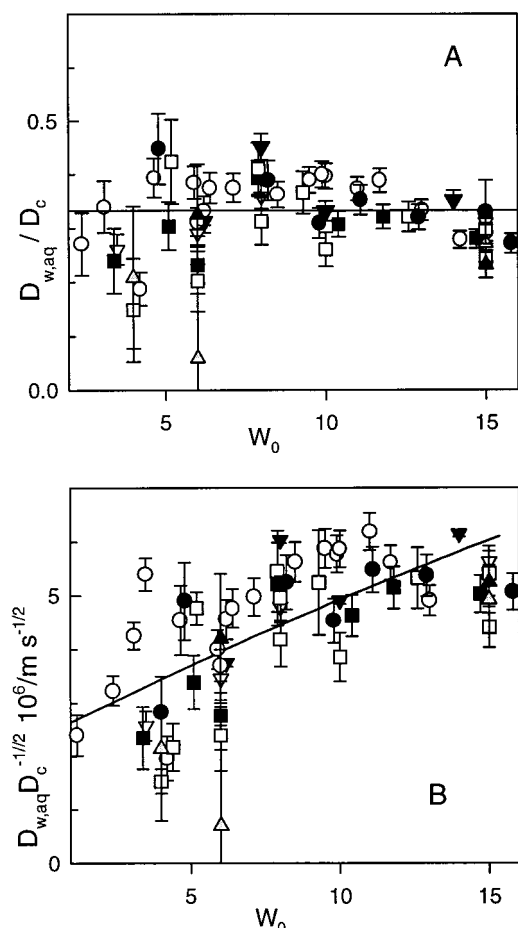(32) Morié, N.; Urbach, W.; Langevin, D. *Phys. Rev. E* **1995**, *51*, 2150.

Figure 7. (A) $D_{w,aq}/D_c$ vs W_0 for different PC concentrations (symbols as in Figure 5). The $D_{w,aq}$ values of Figure 5 were divided by the D_c values (second-order polynomials interpolation of the data of ref 24) at the same W_0. The solid line represents the value $^1/_3$ for $(2D_c\tau)^{1/2} \leq \lambda$. (B) $D_{w,aq}/D_c^{1/2}$ vs W_0 for different PC concentrations. The solid line represents the prediction $D_c^{1/2}/3$ for $(2D_c\tau)^{1/2} \leq \lambda$.

$D_{w,aq}/D_c$ and $D_{w,aq}/D_c^{1/2}$ as function of W_0 in the range $0 < W_0 < 16$ together with the prediction for $\lambda \geq (2D_c\tau)^{1/2}$ (represented by lines). Higher W_0 values were not included because the water diffusion in these cases is governed by the rod-to-sphere transition.[8] Figure 7A shows that the $D_{w,aq}/D_c$ values are almost constant with a weighted average value 0.322 in very good agreement with the prediction ($^1/_3$) for $\lambda \geq (2D_c\tau)^{1/2}$. The plot of Figure 7B shows a further proof for this prediction with the expected dependence on $D_c^{1/2}$. This means that the water molecules do not experience more than one λ before to migrate on another micelle.

This permits us to evaluate an upper limit for τ, because $(2D_c\tau)^{1/2} \leq \lambda$. Using a value[33] of $\lambda \approx 200$ Å and the values[28] of D_c, we obtain $\tau = 1.6{-}0.6$ μs (depending on W_0).

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(33) SANS studies are consistent with a persistence length of 110 Å. However, we used in the calculation a larger statistical length since our results on PC diffusion suggest longer λ.

In the range of C_{PC} explored the static correlation length ξ_s is W_0 independent and almost concentration independent, and it is of the order of λ.[4] As a consequence, an exchange mechanism based only on the migration of water through the entanglement points is unlikely, and we conclude that in addition there is a water exchange between micelles occurring via the bulk solvent. Both these mechanisms may contribute significantly to the macroscopic transport of water, but on the basis of our data, it is difficult to say what could be the dominating process.

Conclusion

Water diffusion has been investigated in a wide range of composition for the system PC/water/cyclohexane. The analysis of the diffusion data collected along oil dilution lines fully supports the model previously proposed by one of us for the contribution of water solubilized in the continuous phase.[23] Thus, it was possible to determine the water diffusion in the aggregates, $D_{w,aq}$. For all the compositions, $D_{w,aq}$ values fall on the same bell-shaped curve when plotted vs W_0. The ascending side of the curve is due to water diffusion inside the aggregates mediated by an intermicellar exchange. Accordingly, the raise in water diffusion reflects a raise in the water mobility (D_c) due to an increase in the fraction of water weakly bound to the PC polar heads. The drop in $D_{w,aq}$ for $W_0 > 15$ is related to the evolution of the aggregates toward small spherical reverse micelles previously reported by us.[8] A comparison between our data and the values of water diffusion in lamellar phases suggests a residence time of the water of about 1 μs in a (cylindrical) reverse micelle. A characteristic time of microseconds for water jumps across a PC interfacial film is fully consistent with the water permeability coefficients reported for bilayers of PC ($10^{-6}{-}10^{-5}$ m s^{-1}).[34] As a final remark, it should be noticed that the characteristic time for the lecithin flip-flop motion is of the order of hours (if not days).[35] This suggests that the transfer of a PC polar head in an hydrophobic environment is severely hindered from a kinetic point of view. This kinetic selectivity on permeate through a PC interfacial layer is likely the origin of the different diffusional behavior of water (fast exchange) and lecithin (slow exchange).

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(34) Tien, H. T. *Bilayer Lipid Membranes*; Marcel Dekker: New York, 1974; Chapter 6 and references therein. Finkelstein, A. *Water Movement Through Lipid Bilayers, Pores and Plasma Membranes*; Wiley-Interscience: New York, 1987; and refences therein.

(35) Devaux, P. F. *FEBS Lett.* **1988**, *234*, 8. Ben-Shaul, A. In *Structure and Dynamics of Membranes*; Lipowsky, R., Sackmann, E., Eds.; Elsevier: Amsterdam, 1995; Vol. 1A, Chapter 7, and references therein.