Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

NMR self-diffusion measurements in inverse micellar cubic phases

Y. Hendrikx[a]; P. Sotta[a]; J. M. Seddon[b]; Y. Dutheillet[a]; E. A. Bartle[c]
[a] Laboratoire de Physique des Solides associé au CNRS, Btiment 510, Université Paris Sud, Orsay, France [b] Department of Chemistry, Imperial College, London, England [c] Department of Chemistry, University of Southampton, Southampton, England

To cite this Article Hendrikx, Y. , Sotta, P. , Seddon, J. M. , Dutheillet, Y. and Bartle, E. A.(1994) 'NMR self-diffusion measurements in inverse micellar cubic phases', Liquid Crystals, 16: 5, 893 — 903
To link to this Article: DOI: 10.1080/02678299408027860
URL: http://dx.doi.org/10.1080/02678299408027860

LIQUID CRYSTALS, 1994, VOL. 16, NO. 5, 893–903

NMR self-diffusion measurements in inverse micellar cubic phases

by Y. HENDRIKX*†, P. SOTTA†, J. M. SEDDON‡, Y, DUTHEILLET† and E. A. BARTLE§

† Laboratoire de Physique des Solides associé au CNRS, Bâtiment 510, Université Paris Sud, 91405 Orsay, France

‡ Department of Chemistry, Imperial College, Exhibition Road, London SW7 2AY, England

§ Department of Chemistry, University of Southampton, Southampton SO9 5NH, England

(*Received 3 August 1993; accepted 29 September 1993*)

We have measured self-diffusion coefficients of amphiphile and water molecules in novel inverse micellar lyotropic cubic phases using the pulsed field gradient NMR technique. We investigated two different ternary lyotropic systems: oleic acid/sodium oleate/water, and dioleoylglycerol/dioleoylphosphatidylcholine/water. Both of these systems have previously been shown by one of us to form a cubic phase of space group Fd3m, whose structure is a complex packing of two types of disconnected quasi-spherical inverse micelles embedded in a 3D hydrocarbon matrix. The amphiphile translational diffusion coefficients determined for the first time by ^{1}H NMR in both systems are surprisingly large. Thus the self diffusion coefficients of amphiphiles may not provide a reliable way of distinguising inverse micellar from inverse bicontinuous phases. The water self-diffusion coefficient has been determined to have a value of $2{\cdot}4 \times 10^{-12}\,m^2\,s^{-1}$, a value which is more than two orders of magnitude lower than that typically observed for inverse bicontinuous cubic phases. This confirms unambiguously the inverse micellar topology of the Fd3m cubic phase, and indicates that the value of the water diffusion coefficient should permit inverse micellar and inverse bicontinuous structures to be reliably distinguished, even for systems where the structure has not been previously determined by diffraction.

1. Introduction

There are four principal locations in the phase diagrams of amphiphile/water systems where lyotropic cubic phases may be found (for recent reviews, see [1–4]). Normal (oil in water) topology cubic phases may be found either adjacent to the micellar solution, often between the normal micellar solution (L_1) and the normal hexagonal (H_1) phase, or between the H_1 phase and the lamellar L_α phase. In the former case the structures probably consist of anisotropic micellar aggregates. In the latter case the structures appear invariably to be bicontinuous [5–10]. Cubic phases may also be found between the L_α and inverse hexagonal (H_{II}) phases. In this case, the structures are inverse (water in oil), and are also invariably bicontinuous. A fourth type of cubic phase, located between the H_{II} and the inverse micellar solution (L_2), was first discovered in a lipid extract from Pseudomonas fluorescens [11]. Different binary amphiphile/water mixtures were shown to exhibit this same cubic phase [12–14]. One of these systems is a binary lipid mixture in water, dioleoylphosphatidylcholine

* Author for correspondence.

0267–8292/94 $10·00

(DOPC)/dioleoylglycerol (DOG)/water, while another consists of sodium oleate (NaO)/oleic acid (OA)/water [12–13]. The authors proposed, from a crystallographic analysis, that this phase is of space group Fd3m (number 227) and that the structure is a packing of two types of disjoined micelles of type II (water in oil), quasi-spherical in shape, embedded in a hydrocarbon 3D matrix [10, 15, 16]. This structure has been confirmed by a quantitative freeze–fracture electron microscopy study [17]. Essentially this structure had previously been predicted as a possible cubic phase structure, on the basis of formal, purely geometric arguments [8].

Self-diffusion coefficient values, either of amphiphile molecules or of water, are directly related to the topology of the corresponding medium, either the hydrophobic one or the aqueous one. Indeed in bicontinuous structures, diffusion is possible over macroscopic distances (1–20 μ) while in structures consisting of closed aggregates diffusion is restricted to the size of the aggregates (~ 50 Å).

Although amphiphile self-diffusion coefficients can clearly distinguish between micellar and bicontinuous phases for the normal topology (oil in water) structures [18–21], it is by no means clear whether this will be true for the corresponding inverse (water in oil) structures. Whilst inverse bicontinuous phases have lipid diffusion coefficients which are similar to those of fluid lamellar phases [22], the situation with regard to inverse micellar cubic phases is much less certain. The only measurements so far reported were of fluorescent lipid analogues in an Fd3m cubic phase of a monoolein/oleic acid/water mixture, using a FRAP (fluorescence recovery after photobleaching) technique [14]. The diffusion coefficients obtained ranged from 0·06 to 7·1 $\times 10^{-12}$ m^2 s^{-1}, depending on the hydrophilicity of the fluorescent lipid probe used. The latter value is as high as the values observed for lipid in inverse bicontinuous phases.

In the present work, we have produced cubic samples of DOG/DOPC/water and OA/NaO/water in the absence of an excess of water phase, and confirmed by X-ray diffraction that the Fd3m structure was obtained in both cases. We have then used the pulsed field gradient NMR technique to measure the self diffusion coefficients of both the amphiphilic molecules and the water in the phase.

The water self-diffusion is found to be extremely slow, in perfect concordance with the topology of the phase. However, the amphiphile diffusion is faster than expected. It is found to be comparable to that in bicontinuous phases of similar molecules.

2. Experimental

2.1. *Samples*

2.1.1. *Oleic acid/sodium oleate/water*

Oleic acid(*cis*-octadec-9-enoic acid) (OA) and sodium oleate (NaO) were of commercial origin (Sigma, purity ~ 99 per cent). Heavy water (D_2O, 99·8 per cent) was provided by 'Service des molécules marquées', CEA-Saclay. Light water (H_2O) was distilled and cleared from any fatty impurity by running it through a Millipore apparatus. The samples were prepared in 10 mm or 5 mm NMR tubes by weighing together known amounts of both surfactants, typically 65 w% OA and 35 wt% NaO. The tubes were left at 40°C for a few days to allow complete mixing of the components. When solubilization of NaO in OA was achieved, the appropriate quantity of heavy water (D_2O) or light water was added, in order to obtain hydrated soap/acid mixtures without any excess of water. The tubes were further stored at 40°C until perfect homogenization was obtained. Compositions of the samples are listed in the table.

Composition of the samples in weight per cent and diffusion coefficients of the different compounds.

Sample				
Number	Compound(s)	wt%	T/°C	$D \times 10^{11}$/m^2 s^{-1}
1	OA	100	40	6·15 ± 0·05†‡
2	OA	56·0	40	1·05 ± 0·05†‡
	NaO	30·0	40	1·05 ± 0·05†‡
	D_2O	14·0	40	—
3	OA	51·0	22	0·45 ± 0·05‡
	NaO	28·0	22	0·45 ± 0·05‡
	H_2O	21·0	22	0·24 ± 0·02‡
4	DOG	100	30	1·04 ± 0·04†‡
5	DOG	58·0	30	~0·64†
	DOPC	25·0	30	~0·09†
	D_2O	17·0	30	—
6	DOG	60·0	30	0·49 ± 0·02‡
	DOPC	26·0	30	0·16 ± 0·03‡
	D_2O	14·0	30	—

† Experimental device I.
‡ Experimental device II.

2.1.2. *Dioleoylglycerol/dioleoylphosphatidylcholine/water*

1,2-Dioleoylglycerol (1,2-di[*cis*-octadec-9-enoyl]glycerol) (DOG) and dioleoylphosphatidylcholine (DOPC) were of commercial origin (Sigma, purity respectively ~98 per cent and 99 per cent). Dioleoylphosphatidylcholine was supplied as 0·025 M chloroform solution. The samples were prepared by first introducing an appropriate volume of the DOPC solution into 10 mm or 5 mm NMR tubes. The solution was evaporated under argon flow, desiccated under vacuum, and the amount of DOPC was weighed. Appropriate amounts of DOG were then added so that the components were in the ratio, 70 wt% DOG, 30 wt% DOPC, and these mixtures were redissolved in chloroform/methanol. When mixing of the components was complete, the samples were evaporated and desiccated again, then weighed. The samples were considered fully desiccated when their weights were equal to that of pure DOG added to pure DOPC (without solvent). At this stage, the samples looked like transparent, homogeneous, isotropic, highly viscous fluids. The appropriate amount of heavy water (D_2O) was then added, in order to obtain fully hydrated DOG/DOPC mixtures without excess of water, as in the case of the OA/NaO/water mixtures, described above. The tubes were then left at room temperature until perfect homogenization was obtained.

At the end of the processes described in §§ 2.1.1 and 2.1.2, the samples were perfectly transparent, isotropic and macroscopically solid. However sample 3 presented some small pasty, milky inhomogeneities.

2.2. *X-Ray diffraction experiments*

The structures of polycrystalline samples were obtained by small angle X-ray diffraction. We used a Guinier camera with linear collimation, monochromatic Cu$K\alpha_1$ radiation (wavelength, $\lambda = 1{\cdot}5405$ Å) and a sample–film distance equal to 123·3 mm.

The experiments were carried out either at 40 ± 1°C or at 30 ± 1°C. The samples were held between mica sheets separated by a teflon spacer (thickness 0·7 mm) in a cell whose tightness was ensured by a set of joints and mechanical clamping.

2.3. *Pulsed field gradient NMR experiments—Self diffusion measurements*

Translational diffusion coefficients in numerous amphiphilic systems have been measured, and particularly in the cubic phases [18–20, 23–27]. The amphiphile diffusion in micellar cubic phases, both direct and inverse, is expected to be much slower than in bicontinuous ones. Water diffusion is expected to be highly restricted in inverse micellar phases only, since in any other case, the aqueous medium is continuous.

The diffusion coefficients were determined by the standard proton (^{1}H) pulsed field gradient (PFG) NMR technique [28]. A spin echo is produced by a 90°–180° radiofrequency pulse sequence. A field gradient G is applied during a time δ, after each radiofrequency pulse. In the case that only one diffusing species is present, the ratio of the echo amplitudes A_G and A_0, with and without field gradient respectively, is given by [29]

$$\ln(A_G/A_0) \sim -\gamma^2 G^2 D \delta^2 (\Delta - \delta/3), \tag{1}$$

where γ is the magnetogyric ratio for the proton ($\gamma = 2{\cdot}6753 \times 10^8\ s^{-1}\ T^{-1}$), D the self-diffusion coefficient and Δ the time interval between the gradient pulses. The diffusion coefficient, D, may be determined by varying either the time intervals or the gradient magnitude. The duration Δ corresponds typically to a diffusion length of the order 1 μm. Formula (1) is valid if the diffusion is not limited to regions smaller than this typical distance (1 or a few μm). The field gradient amplitude G is calibrated with a known diffusion coefficient D_0, namely that for glycerol at 40°C ($D_0 = 6 \times 10^{-12}\ m^2\ s^{-1}$) [30].

In a first series of experiments, a Brüker Minispec, operating on protons at 20 MHz with a permanent magnet, was used, together with a Brüker pulsed magnetic field gradient unit (device *I*). The in-phase u and quadrature v components of the preamplified signal were obtained with a Brüker phase detector/amplifier, and then digitized. The amplitude of the signal was computed as $(u^2+v^2)^{1/2}$ and then accumulated 10 to 50 times in order to increase the signal/noise ratio. This procedure eliminates the fluctuations in the echo phase. In this experiment, G was kept constant ($2{\cdot}5\ T\,m^{-1}$) and the time intervals δ and Δ varied between 0·5 and 6, and 12 and 100 ms, respectively. In that range of Δ values, the echo shape was checked to be the same with and without gradient pulses (even at high G values). This condition fixed the shorter accessible value for Δ (12 ms).

Experiments were also carried out with a CXP 100 Brüker spectrometer operating at 90 MHz, equipped with a Drusch electromagnet of 2·1 T and a homemade pulsed magnetic field gradient unit (device *II*). The echo was accumulated in the same way as described above. The ratio $\ln A_G/A_0$ was measured as a function of G (between 0·5 and $6\ T\,m^{-1}$), or as a function of the quantity $\delta^2(\Delta - \delta/3)$, with δ varying between 1·5 and 6 ms, and Δ between 40 and 100 ms. This device also allows one to perform medium resolution Fourier transform experiments: in FT PFG experiments, the spin echo decay is Fourier transformed to give frequency resolved information on the diffusion of the species present in the system. In that case the signal was not accumulated, the signal to noise ratio being high enough to work on single shot spectra.

3. Results

3.1. *X-Ray diffraction experiments*

Diffraction patterns were obtained for the OA/NaO/D_2O and DOG/DOPC/D_2O systems at 40°C and 30°C (data not shown). The diffraction patterns of the Fd3m cubic phases gave 10 and 11 Bragg reflections, respectively. The indexing of the reflections was assessed by plotting the reciprocal spacings, $q_{hkl}/2\pi$, of the observed Bragg reflections versus $(h^2+k^2+l^2)^{1/2}$ according to the relation $q_{hkl}/2\pi = 1/a(h^2+k^2+l^2)^{1/2}$ with a being the cubic lattice parameter. This plot as Fd3m is shown for the OA/NaO/D_2O system in figure 1. The X-ray diffraction patterns are identical to those already published [10, 12, 15, 16]. The spottiness in both diffraction patterns indicated the presence of a rather limited number of monodomains of the cubic phase in the samples, which suggests that their size may be rather large on average.

The lattice parameter, a is $119{\cdot}7 \pm 1{\cdot}8$ Å, for the OA/NaO/D_2O (table, sample 2) cubic phase and $149{\cdot}9 \pm 5{\cdot}2$ Å for the DOG/DOPC/D_2O (table, sample 5) cubic phase.

3.2. *NMR self diffusion measurements*

3.2.1. *Amphiphile diffusion in the OA/NaO/D_2O system*

The results of the measurements are given in the table.

The diffusion curves $\ln A_G/A_0$ versus $\delta^2(\Delta - \delta/3)$ or versus G^2 for the amphiphile molecules of OA/NaO/D_2O are straight lines. They were obtained without Fourier transformation, the ratio A_G/A_0 being measured on the echo amplitude itself. According to formula (1) this result means that no restricted diffusion occurs in the system and that one single diffusion coefficient is observed for both entities (OA and NaO), with $D = 1{\cdot}05 \pm 0{\cdot}05 \times 10^{-11}$ m^2 s^{-1}. This is related to the fact that OA and NaO differ only by the cations (H^+ or Na^+), which are obviously in fast exchange with respect to the time scale involved in the PFG NMR experiment (10 to 100 ms). The

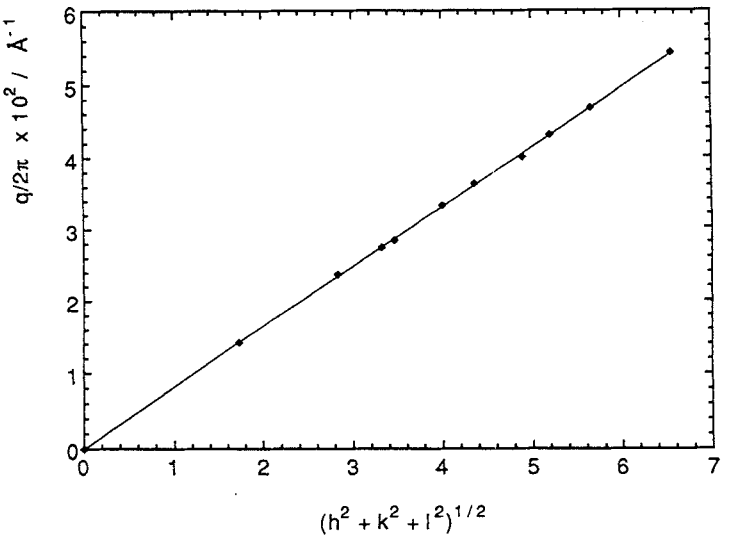

Figure 1. Reciprocal spacings, $q/2\pi$, of the reflections versus $(h^2+k^2+l^2)^{1/2}$, for the system OA/NaO/D_2O (sample 2).

system then behaves like a pseudo binary (single amphiphile/brine) system, and one single 'average' amphiphile molecule is observed. The diffusion coefficient of pure OA has also been measured; it is equal to $6{\cdot}15 \pm 0{\cdot}05 \times 10^{-11}\,m^2\,s^{-1}$ at the same temperature.

3.2.2. *Amphiphile diffusion in the DOG/DOPC/D_2O system*

In this case the amphiphile molecules (DOG and DOPC) are quite different, and may be expected to have different diffusion coefficients. The diffusion curves $\ln A_G/A_0$ versus $\delta^2(\Delta - \delta/3)$, shown in figures 2 (*a*) and (*b*), are clearly non-linear, in contrast to the OA/NaO/D_2O case. The gradient magnitude was also varied while keeping the time

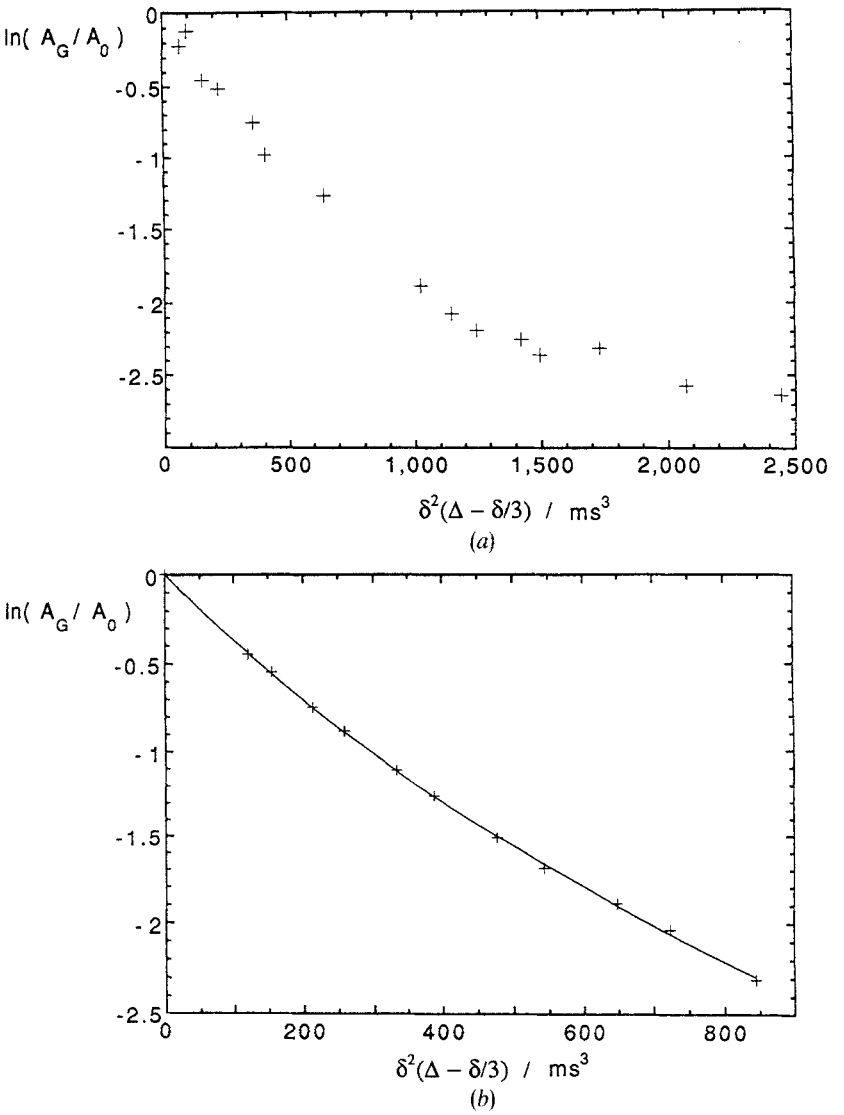

Figure 2. Diffusion curve, $\ln A_G/A_0$ versus $\delta^2(\Delta - \delta/3)$, obtained for the DOG/DOPC/D_2O cubic phase: (*a*), sample 5, experimental device *I*. (*b*), sample 6, experimental device *II*. Diffusion data are fitted according to formula (4).

intervals constant. The curve $\ln A_G/A_0$ versus G^2 is shown in figure 3: it is also non-linear. It is likely that no restricted diffusion occurs in this system, as in the case of the OA/NaO/D_2O system (over regions of a few μm, as mentioned in § 2.3), and that the non-linearity in the diffusion curves may result from the presence of two molecules with different diffusion coefficients. In order to distinguish the two molecules, a straightforward way should be to use FT experiments. However, in practice it was not possible to distinguish the two amphiphile molecules, since at long time intervals, the echo signal is dominated by the hydrophobic chains which are identical in both molecules (DOG and DOPC).

In the case where two molecules with different diffusion coefficients are present, which cannot be resolved by FT methods, formula (1) has to be replaced by the following expression:

$$\frac{A_G}{A_0}=\frac{\Phi\exp(-\gamma^2G^2\tau D_a)\exp(-t/T_{2a})+(1-\Phi)\exp(-\gamma^2G^2\tau D_b)\exp(-t/T_{2b})}{\Phi\exp(-t/T_{2a})+(1-\Phi)\exp(-t/T_{2b})}, \quad (2)$$

where the simplified notation $\tau=\delta^2(\Delta-\delta/3)$ has been used; Φ is the molar (proton) fraction of species a (say, DOG), i.e. the relative number of protons belonging to molecules a. t is the time interval between radiofrequency pulses.

If care is taken to work at constant t, (2) simplifies to

$$\frac{A_G}{A_0}=\Phi'\exp(-\gamma^2G^2\tau D_a)+(1-\Phi')\exp(-\gamma^2G^2\tau D_b), \quad (3)$$

where Φ' includes both Φ and the T_2 factors. If T_{2a} and T_{2b} are close together, Φ' is expected to be close to the actual fraction Φ.

It is clear that the fastest diffusion coefficient must be attributed to DOG, whose polar head group is much less hydrophilic than that of DOPC. In figures 2 (*b*) and 3 the diffusion data are fitted using formula (3). Both series of data may be satisfactorily fitted with the same adjustable parameters Φ', D_{DOG} and D_{DOPC}. Φ' is found to be $0{\cdot}69\pm0{\cdot}02$, remarkably close to the actual compositional value of the samples. The

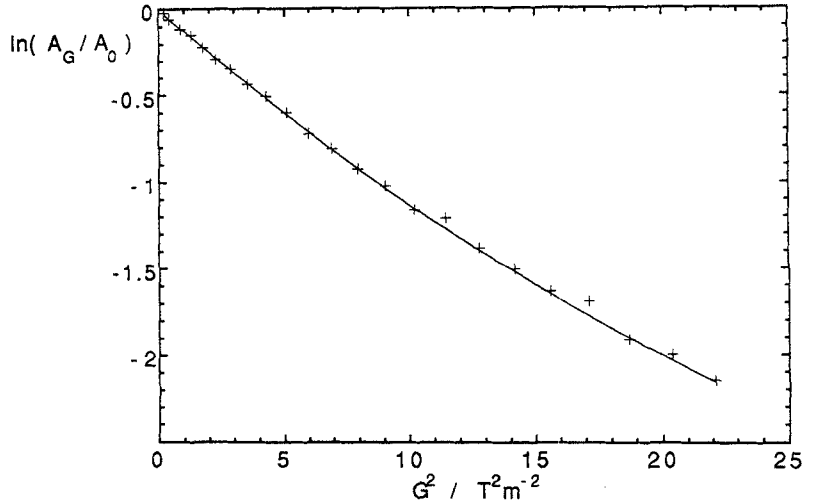

Figure 3. Diffusion curve, $\ln A_G/A_0$ versus G^2, obtained for the DOG/DOPC/D_2O cubic phase. Diffusion data are fitted according to formula (4). Sample 6, experimental device *II*. The time intervals are $\delta=3$ ms and $\Delta=53{\cdot}1$ ms.

diffusion coefficients extracted from the fits are $D_{DOG} = 4{\cdot}9 \pm 0{\cdot}15 \times 10^{-12}\,m^2\,s^{-1}$ and $D_{DOPC} = 1{\cdot}6 \pm 0{\cdot}3 \times 10^{-12}\,m^2\,s^{-1}$.

In figure 2(*a*), the T_2 factors were not kept constant. Therefore, these data were not used to extract diffusion coefficients. The values given in the table (sample 5) are estimated from the slopes at short and long time intervals, based on the assumption that T_2 values for both amphiphile species are similar.

The diffusion coefficient of pure DOG was measured to be equal to $1{\cdot}04 \pm 0{\cdot}04 \times 10^{-11}\,m^2\,s^{-1}$.

3.2.3. *Water diffusion in the OA/NaO/H_2O system*

In order to measure the water self-diffusion coefficient, a fully protonated sample was prepared and Fourier transformation was used to discriminate the amphiphile and water signals. The experiment was carried out at 22°C. The durations were $\delta = 2{\cdot}17$ ms and $\Delta = 63{\cdot}87$ ms. Typical spectra, obtained for different gradient amplitudes (between 0 and 7 Tm^{-1}), are shown in figure 4. The spectra exhibit two well-resolved lines; the up-field shifted line belongs to water, and the other one to the protons in the hydrophobic chains of the amphiphilic molecules. The diffusion coefficients extracted from these spectra are $D_{OA} = 4{\cdot}5 \times 10^{-12}\,m^2\,s^{-1}$ for the amphiphiles and $D_w = 2{\cdot}4 \times 10^{-12}\,m^2\,s^{-1}$ for the water.

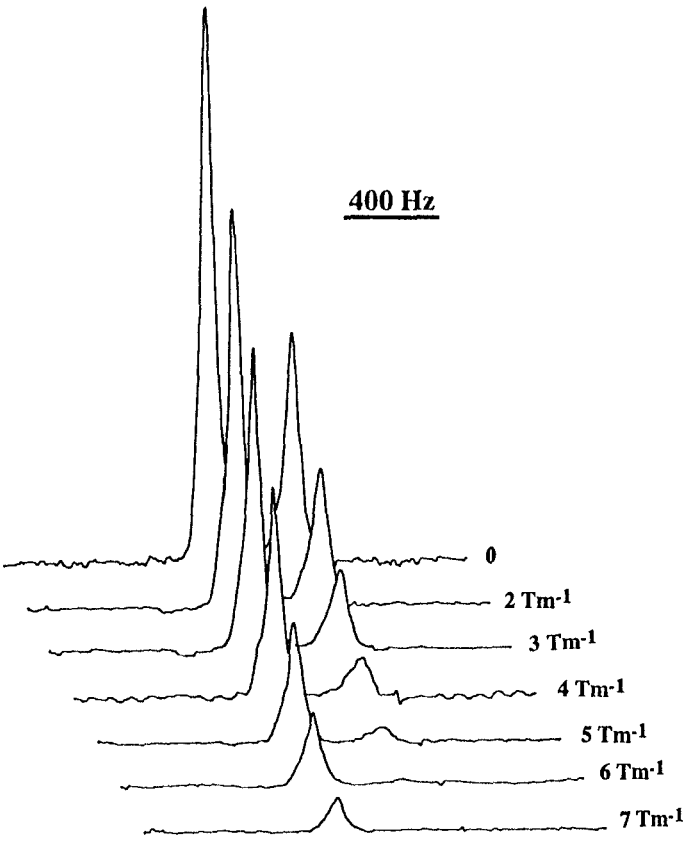

Figure 4. Spectra obtained by Fourier transforming the right hand side of the echo, in the OA/NaO/H_2O cubic phase (sample 3, device *II*). The durations are $\delta = 2{\cdot}17$ ms, $\Delta = 63{\cdot}87$ ms. The gradient amplitudes are indicated.

4. Discussion

4.1. *NMR self diffusion measurements*

For the bicontinuous cubic phase of space group Ia3d, of simple single chain surfactant systems, the value of D for amphiphile molecules is typically 10^{-11} to $10^{-10}\,m^2\,s^{-1}$ [18–20], whereas similar systems such as dodecyltrimethylammonium chloride ($C_{12}TACl$) which form a direct micellar (oil in water) cubic phase, of space group Pm3n, have amphiphile self-diffusion coefficients which are 50 to 100 times lower, typically 10^{-13} to $10^{-12}\,m^2\,s^{-1}$ [18, 20, 21].

For phospholipids (single-(lyso) or double-chained), the value of D in the bicontinuous cubic phases, whether of normal or inverse topology, is in the range $2\text{–}8\times10^{-12}\,m^2\,s^{-1}$, i.e. is comparable to that observed in the corresponding lamellar phases.

For both bicontinuous cubic and lamellar phases, the water self-diffusion coefficients, D_w, are in the region $5\times10^{-10}\,m^2\,s^{-1}$, i.e. approximately four times smaller than in bulk water, which is $2{\cdot}26\times10^{-9}\,m^2\,s^{-1}$ at 25°C [30]. The values of D_w for cubic phases composed of normal micellar aggregates should be quite similar, but should be very low for cubic phases composed of inverse micellar aggregates. As expected, the water self-diffusion coefficient, $D=2{\cdot}4\times10^{-12}\,m^2\,s^{-1}$, in the $OA/NaO/H_2O$ system (table, sample 3), is more than two orders of magnitude smaller than in bicontinuous phases ($5\times10^{-10}\,m^2\,s^{-1}$).

The diffusion coefficients of the amphiphilic molecules in the Fd3m cubic phase, measured in both of our systems, are larger than we expected. There is an apparent discrepancy between the diffusion coefficients we have measured and those obtained for the mono-olein/oleic acid/water Fd3m cubic phase by FRAP when charged or phospholipid fluorescent probes were used [14]. The authors pointed out that the diffusion coefficient of the fluorescent lipid in the investigated system is probe dependent and noted that it increases dramatically with decreasing water affinity of the polar head group of the probe. When the hydrophilic–hydrophobic balance was shifted towards being less polar, the probe traverses the intermicellar gap more easily and the diffusion coefficient was found to increase from $6\pm0{\cdot}02\times10^{-14}\,m^2\,s^{-1}$ for a charged probe to $7{\cdot}1\pm0{\cdot}8\times10^{-12}\,m^2\,s^{-1}$ for a fatty acid probe.

Let us first consider the case of the OA/NaO/water system. The single observed diffusion coefficient ($D=1{\cdot}05\pm0{\cdot}05\times10^{-11}\,m^2\,s^{-1}$) is of the order of the diffusion coefficients observed for bicontinuous cubic phases of single chain surfactants. The influence of grain boundaries or defects is assumed to be negligible, due to the presence or relatively large monodomains of cubic phase in the investigated samples. A typical exchange time t_{ex} between micelles may be roughly estimated, using the equation $\langle d^2\rangle=2Dt_{ex}$, where d is the distance between neighbouring micelles. With d of the order of 50 Å [32], this gives $t_{ex}\sim10^{-7}$ to 10^{-6} s. Though quite short, this value is not completely unreasonable, and comparable to that given in [14] in the case of the less hydrophilic fluorescent probe. For comparison, phopholipid flip–flop in pure lipid bilayers is a slow process occurring on a timescale of hours to weeks, depending on the particular lipid species [33]. This discrepancy has been discussed by Cribier *et al.* [14]. The diffusion coefficient is only six times lower than for neat oleic acid. This implies that at a given instant, a significant fraction of the molecules are 'free', i.e. not anchored at the amphiphile/water interface. In that case the measured long-range diffusion coefficient results from an average of diffusion within the 3D hydrocarbon matrix and diffusion due to micelles. The first contribution (coefficient D_{free}) is that of the molecules which diffuse from one micelle to another and behave at a given instant as 'free'

molecules. The second contribution (coefficient $D_{micelle}$) may be roughly equated to D_w, if it is assumed that water molecules cannot cross the hydrophobic medium. According to this, we may write that

$$D_{OA/NaO} = \varphi D_{free} + (1-\varphi) D_{micelle}, \quad (4)$$

where φ is the molar (proton) fraction of the 'free' molecules and $(1-\varphi)$ the molar (proton) fraction at the surface of the micelles [34]. As noted above, $D_{micelle} \sim D_w$ and we may consider $D_{free} = \beta D_{OA}$, where β is a geometric obstruction factor. One can assume that $2/3 < \beta < 1$, according to the relation $\beta \sim 1/(1+v/2)$ [27], wherein v is the volume fraction of hydrophilic regions. We have measured the diffusion coefficient of pure OA at 22°C, $D_{OA} = 2{\cdot}85 \pm 0{\cdot}05 \times 10^{-11}\,m^2\,s^{-1}$. We can thus estimate φ at 22°C for our sample composition to be in the range $0{\cdot}1 < \varphi < 0{\cdot}15$.

Knowing the cell parameter and the composition of the sample, we have estimated the available area per polar head group, $S \sim 16\,Å^2$, which is low compared to that which should be occupied by a fully extended saturated hydrocarbon chain with a polar carboxylic head group (in a condensed state) at the water/air interface (18–19 $Å^2$) [35]. If the same estimate is made, assuming $0{\cdot}1 < \varphi < 0{\cdot}15$, we calculate that S has a value which varies between 17·8 and 18·8 $Å^2$, which is quite reasonable.

However neat OA molecules may be self-associated, whereas in the hydrocarbon region they may well be monomers. In that case the diffusion of OA in the hydrocarbon region might be much faster than that of neat OA and the 'free' fraction φ is overestimated.

Also in the DOG/DOPC/water system the extracted diffusion coefficients are of the order of those in bicontinuous cubic phases of double-chained amphiphile molecules. Moreover, if we consider that all the amphiphile molecules are anchored at the amphiphile/water interface, we found that the mean area available per polar group is $\sim 35\,Å^2$, which seems rather small for a double-chained unsaturated molecule and the polar heads involved, in particular the DOPC one [36]. This suggests that also in this system a fraction of molecules are 'free' within the 3D hydrocarbon matrix. It is clear that mainly DOG molecules will be 'free', being much less hydrophilic than DOPC ones. Therefore, neglecting the fraction of free DOPC molecules, one could estimate a fraction of free DOG molecules $\varphi \sim 0{\cdot}3$. Note that applying equation (4) to the diffusion of the DOG molecules, knowing the diffusion coefficient of pure DOG to be $1{\cdot}04 \pm 0{\cdot}04 \times 10^{-11}\,m^2\,s^{-1}$ and assuming $D_{micelle} \sim D_{DOPC}$ would lead to a fraction of free DOG molecules in the range $\varphi \sim 0{\cdot}5$, which seems much less reasonable than the value found for the OA/NaO/water system.

5. Conclusions

We have measured the amphiphile diffusion coefficients in two different systems which give the Fd3m (number 227) cubic phase occurring between the inverse hexagonal phase and the inverse micellar solution, which consists of closed inverse micellar aggregates. The diffusion coefficients of the amphiphile molecules obtained with the ^1H PFG NMR technique on both systems are higher than we expected. However, the diffusion coefficient of water is more than two orders of magnitude smaller than that found in bicontinuous cubic phases and corroborates the identified structure and topology. The fact that the diffusion coefficient of the amphiphile molecules is higher than expected (based on previous diffusion studies of direct oil in water micellar cubic phases) may be explained by noting that the hydrophobicity of the molecules is larger than their hydrophilicity and therefore the molecules diffuse more easily from one micelle to another when the 3D matrix is a hydrocarbon one than in the

case of a direct cubic micellar phase where the 3D matrix is a hydrophilic one. The water self-diffusion coefficient should be measured in order to distinguish reliably an inverse micellar cubic phase from a bicontinuous one.

The pulsed field gradient unit for the Brüker CXP 100 spectrometer was built by R. Andreani and W. Gorecki from the 'Laboratoire de Spectrométrie Physique' of the University of Grenoble. Discussions with B. Chapellier from the 'Laboratoire de Physique des Solides d'Orsay' are gratefully acknowledged. We acknowledge the financial support of 'programme ALLIANCE' for British–French cooperation.

References

[1] LINDBLOM, G., and RILFORS, L., 1989, *Biochim. biophys. Acta*, **988,** 221.
[2] LARSSON, K., 1985, *Colloid Polym. Sci.*, **268,** 264.
[3] FONTELL, K., 1990, *Colloid Polym. Sci.*, **268,** 264.
[4] SEDDON, J. M., 1990, *Biochim. biophys. Acta*, **1031,** 1.
[5] BALMBRA, R. R., CLUNIE, J. S., and GOODMAN, J. F., 1969, *Nature, Lond.*, **222,** 1159.
[6] FONTELL, K., FOX, K., and HANSSON, E., 1985, *Molec. Crystals liq. Crystals Lett.*, **1,** 9.
[7] ERIKSSON, P. O., LINDBLOM, G., and ARVIDSON, G., 1985, *J. phys. Chem.*, **89,** 1050.
[8] CHARVOLIN, J., and SADOC, J. F., 1988, *J. Phys., Paris*, **49,** 521.
[9] LUZZATI, V., 1968, *Biological Membranes*, Vol. 1, edited by D. Chapman (Academic Press, London), p. 71.
[10] MARIANI, P., LUZZATI, V., and DELACROIX, H., 1988, *J. molec. Biol.*, **204,** 165.
[11] TARDIEU, A., 1972, Ph.D. Thesis, Université de Paris-Sud.
[12] SEDDON, J. M., 1990, *Biochemistry*, **29,** 7998.
[13] SEDDON, J. M., BARTLE, E. A., and MINGINS, J., 1990, *J. Phys. Condens. Matter*, **2,** SA285.
[14] CRIBIER, S., GULIK, A., FELLMAN, P., VARGAS, R., DEVAUX, F., and LUZZATI, V., 1993, *J. molec. Biol.*, **229,** 517.
[15] MARIANI, P., RIVAS, E., LUZZATI, V., and DELACROIX, H., 1990, *Biochemistry*, **29,** 6799.
[16] LUZZATI, V., VARGAS, R., GULIK, A., MARIANI, P., SEDDON, J. M., and RIVAS, R., 1992, *Biochemistry*, **31,** 279.
[17] DELACROIX, H., GULIK-KRZYWICKI, T., and SEDDON, J. M. (in preparation).
[18] BULL, T. E., and LINDMANN, B., 1974, *Molec. Crystals liq. Crystals*, **28,** 155.
[19] LINDBLOM, G., and WENNERSTRÖM, H., 1977, *Biophys. Chem.*, **6,** 167.
[20] ERIKSSON, P. O., JOHANSSON, B. A., and LINDBLOM, G., 1984, *Surfactants in Solution*, Vol. 1, edited by K. L. Mittal and B. Lindman (Plenum Press), p. 219.
[21] ERIKSSON, P. O., LINDBLOM, G., and ARVIDSON, G., 1987, *J. phys. Chem.*, **91,** 846.
[22] RILFORS, L., ERIKSSON, P. O., ARVIDSON, G., and LINDBLOM, G., 1986, *Biochemistry*, **25,** 7702.
[23] STILBS, P., 1986, *Prog. Nucl. Magn. Reson. Spectrosc.*, **19,** 1.
[24] CHARVOLIN, J., and RIGNY, P. J., 1973, *Chem. Phys.*, **58,** 3999.
[25] LINDBLOM, G., LARSSON, K., JOHANSSON, L., FONTELL, K., and FORSEN, S., 1979, *J. Am. chem. Soc.*, **101,** 5465.
[26] SODERMAN, O., OLSSON, U., and WONG, T. C., 1989, *J. phys. Chem.*, **93,** 7474.
[27] JONSSON, B., WENNERSTRÖM, H., NILSSON, P. G., and LINSE, P., 1986, *Colloid Polym. Sci.*, **264,** 77.
[28] STEJSKAL, E. O., and TANNER, J. E., 1965, *J. chem. Phys.*, **42,** 288.
[29] TANNER, J. E., 1970, *J. chem. Phys.*, **52,** 2523.
[30] TOMLINSON, D., 1972, *J. molec. Phys.*, **25,** 735.
[31] MILLS, R., 1973, *J. phys. Chem.*, **77,** 685.
[32] LUZZATI, V., VARGAS, R., MARIANI, P., GULIK, A., and DELACROIX, H., 1993, *J. molec. Biol.*, **229,** 540.
[33] NICHOLS, J. W., 1993, *Phospholipids Handbook*, edited by G. Cevc (Marcel Dekker), p. 663.
[34] KATO, T., TAKEUCHI, H., and SEIMIYA, T., 1992, *J. phys. Chem.*, **96,** 6839.
[35] HENDRIKX, Y., 1973, *J. Chim. phys.*, **11–12,** 1727.
[36] Indeed the minimal area available for an egg yolk lecithin molecule in a mononolecular film at the water/air interface is 54–56 Å^2. HENDRIKX, Y., and TER-MINASSIAN-SARAGA, L., 1970, *J. Chim. phys.*, **67,** 1620. HENDRIKX, Y., and TER-MINASSIAN-SARAGA, L., 1975, *Advances in Chemistry Series*, Number **144,** 'Monolayers', p. 177.