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INTRAMOLECULAR DISORDER AND ITS RELATION TO MESOPHASE STRUCTURE IN LIPID/WATER MIXTURES

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SUMMARY

NMR spin-half pair dipolar echo measurements are reported for the lamellar (dispersions and multibilayer stacks) and hexagonal phases of potassium palmitate/^2H_2O mixtures. In the lamellar L_β and L_γ (gel) phases the alkyl chains are rigid and perfectly ordered, while in the lamellar L_α and hexagonal phases they are flexible and disordered. In particular, the measurements show that in the fluid lamellar L_α phase the chain is "bent" at the C_9-C_{10} segment; but is "straight" in the hexagonal phase.

INTRODUCTION

NMR spectroscopy is a particularly attractive technique for studying the dynamic structure of lipid/water mixtures and biological membranes. The ^1H and ^{13}C frequency spectra are determined principally by the anisotropic dipole-dipole interactions and the ^2H spectra are dominated by the anisotropic nuclear quadrupolar interactions; this is a consequence of the liquid crystalline character of these systems. The proper treatment of the ^1H-^1H and ^{13}C-^1H dipolar interactions poses a fundamental, but complex, NMR problem [1–5] which has not yet been resolved. In the case of ^{13}C spectroscopy, the ^{13}C-^1H dipolar interactions may, however, be eliminated either by the use of double resonance techniques [6] or by studying small bimolecular vesicles [7]. High resolution spectra can then be observed and used to study the kinetics of segmental motions in the alkyl chains.

Complementary information about segmental order parameters may be obtained from measurements of quadrupolar splittings in the ^2H spectra of selectively deuteriated amphiphiles [8–11]. Studies have been made in the fluid L_α lamellar phase and have demonstrated the existence of a characteristic amphiphilic chain flexibility. In contrast, ^1H NMR spectroscopy has not yet provided any particularly useful structural information for these systems, albeit that protons are the most abundant nuclear species and, for sensitivity reasons, are convenient to study. Furthermore, the technique does not require the synthesis of specifically substituted molecules and is equally applicable to all the phases.

The protons in the alkyl chain are, with the exception of the methyl groups, arranged in chemically equivalent pairs. Their NMR behaviour is, therefore, expected to be characteristic of a system of interacting spin-half pairs [12–16]. This has important consequences for the fundamental interpretation and application of ^1H NMR to lipid/^2H_2O mixtures [18]. In this paper we will be concerned only with the use of the spin-half pair dipolar echo experiment [12–14] to selectively measure the dipolar interactions between methylene proton pairs and thereby obtain information about the conformational disorder of the amphiphiles [15–17]. In particular, measurements are reported for the lamellar L_α (fluid), L_β and L_γ (gel) and hexagonal phases of potassium palmitate/^2H_2O mixtures. It is found that the conformational disorder of the amphiphiles is characteristically related to the supramolecular organization of these phases, while in the lamellar L_β and L_γ phases the chains are in their all-trans configuration, in the fluid L_α phase they are flexible, but "bent" at the C_9-C_{10} segment.

EXPERIMENTAL MEASUREMENTS AND RESULTS

The proton NMR responses to the resonant rf pulse sequences $90°$-τ-$\beta°_{0°}$ (XX) and $90°$-τ-$\beta°_{90°}$ (XY) observed in the lamellar and hexagonal phases of potassium palmitate/^2H_2O mixtures [22, 23] are characteristic of a spin-half pair system [12–17]. Figs 1 and 2 show typical echo responses observed in these phases. The maximum echo amplitudes are

$$E(\tau, \beta)_{XX} = -\tfrac{2}{3}\sin^2\beta\cos\beta \qquad (1)$$

and $E(\tau, \beta)_{XY} = \sin^2\beta + \tfrac{1}{3}\sin^2\beta\cos^2\beta$.

Similar behaviour is also observed in the corresponding phases of potassium laurate/^2H_2O and the ternary system sodium octanoate/octanol/^2H_2O [17].

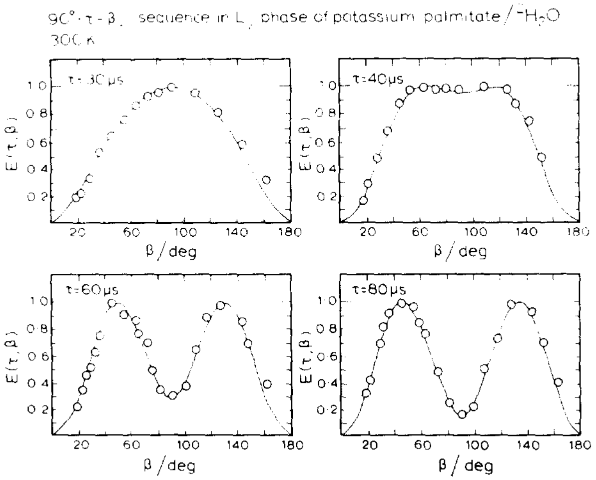

Fig. 1. The dependence of the $90°$-τ-$\beta_{90°}$ maximum echo amplitude $E(\tau, \beta)_{XY}$ on the rotation angle β and the pulse spacing τ for the protons in the lamellar L_γ phase of potassium palmitate/^2H_2O. Each figure represents the variation of $E(\tau, \beta)_{XY}$ with β for fixed τ; the continuous line drawn through the experimental points ○ has been calculated using $E(\tau, \beta)_{XY} = \exp[-\tfrac{1}{2}\{\tfrac{1}{2}M_2(\text{inter})\}\tau^2]\sin^2\beta + \tfrac{1}{3}\exp[-\tfrac{1}{2}\{\tfrac{1}{2}M_2{}^*(\text{inter})\}\tau^2]\sin^2\beta\cos^2\beta$. In each plot the maximum $E(\tau, \beta)_{XY}$ has been normalized to unity. The value of M_2 (inter) was taken from Fig. 7; $M_2{}^*$ was determined experimentally [14, 15] and equal to $1.3 \cdot 10^{-8}$ T^2.

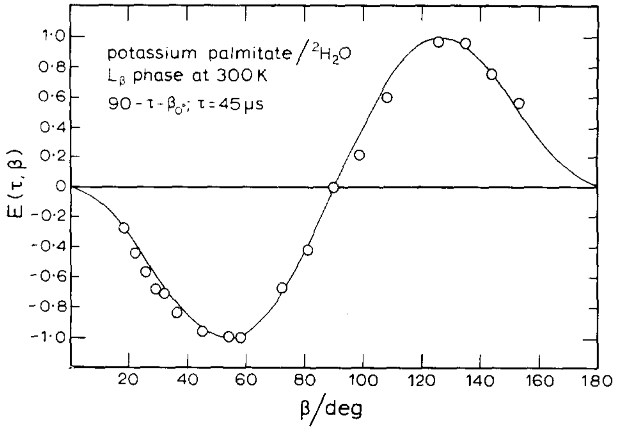

Fig. 2. The dependence of the $90°\text{-}\tau\text{-}\beta_{0°}$ maximum echo amplitude $E(\tau, \beta)_{xx}$ on the rotation angle β for the protons in the L_γ phase of potassium palmitate/^2H_2O mixtures at $\tau = 45\,\mu s$. The continuous line drawn through the experimental points ○ has been calculated using Eqn 1.

The τ dependence of the maximum echo amplitude following a resonant $90\text{-}\tau\text{-}90°_{90°}$ sequence [12, 14] in the L_β and L_γ phases is found to be (Fig. 3) (N.B. Here "lamellar dispersion" denotes an un orientated sample).

$$E(\tau) = E(0)\exp[-\tfrac{1}{2}\{\tfrac{1}{2}M_2(\text{inter})\}\tau^2] \quad (\text{type I}) \qquad (2)$$

where $M_2(\text{inter})$ is the contribution to the second moment of the frequency spectrum from the dipolar interactions between methylene proton pairs. Similar behaviour is also found in the hexagonal phase (Fig. 4) and is analogous to that we have previously reported [15, 16] for classical nematic thermotropic liquid crystals. The behaviour

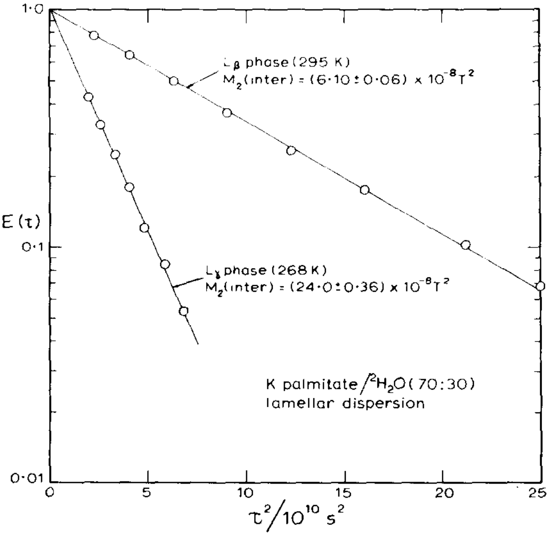

Fig. 3. The dependence of the $90\text{-}\tau\text{-}90°_{90°}$ maximum echo amplitude $E(\tau)$ on the square of the pulse spacing measured in the L_γ and L_β phases of a lamellar dispersion of potassium palmitate/^2H_2O. The straight lines represent the best fit of Eqn 2 to the experimental measurements.

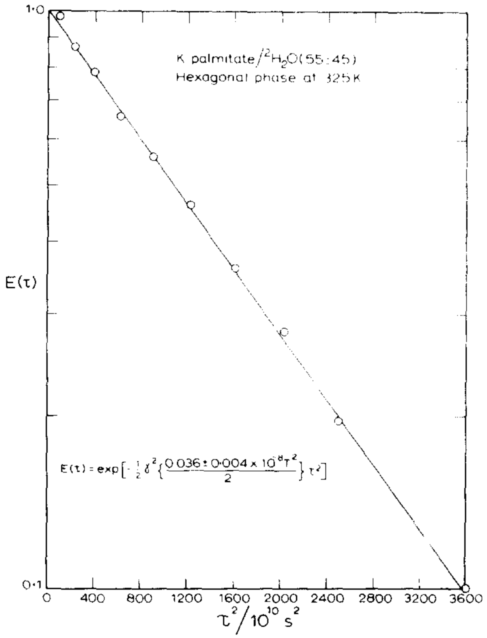

Fig. 4. The dependence of the $90°$-τ-$90°_{90°}$ maximum echo amplitude $E(\tau)$ on the square of the pulse spacing τ measured in the hexagonal phase of potassium palmitate/^2H_2O. The straight line represents the best fit of Eqn 2 to the experimental measurements.

observed in the L_α phase is more complex. Multibilayer stacks oriented between glass plates [19] exhibit an echo decay (Fig. 5) described by

$$\begin{aligned} E(\tau) &= \mathrm{A} \exp\,[-\tfrac{1}{2}\{\tfrac{1}{2}M_2'(\mathrm{inter})\}\tau^2] \\ &\quad + \mathrm{B} \exp\,[-\tfrac{1}{2}\{\tfrac{1}{2}M_2''(\mathrm{inter})\}\tau^2] \qquad \text{(type II)} \end{aligned} \tag{3}$$

with M'_2(inter) > M''_2(inter). The latter quantities exhibit a $(3\cos^2\theta - 1)^2$ dependence on the orientation, θ, of the bilayer normal with respect to the direction of the applied magnetic field. The complex free induction decay signal of these multibilayers exhibits a similar dependence on θ. The corresponding signal from unoriented dispersions of the L_α phase is simpler, being a superposition of Gaussian (two) and Lorentzian (one) components. Similar signals have been reported for lamellar dispersions of potassium laurate/^2H_2O and potassium palmitate/^2H_2O mixtures [1, 2 and 4]. The Lorentzian component has been attributed to the quenching of the dipolar interactions for those molecules whose axes lie close to the magic angle of 54° 44′ relative to the direction of the magnetic field [4]. The Lorentzian component is, therefore, unaffected by the second pulse of the $90°$-τ-$90°_{90°}$ sequence and the dipolar echo decay (Fig. 6) is again described by Eqn 3. But the ratios of M'_2(inter) and M''_2(inter) for the multibilayer ($\theta = 0°$) and the dispersion approximate the factor of 5 predicted for a system of randomly oriented lamellae. Measurements of interpair second moments may thus be made in either dispersions or multibilayer stacks.

The values of the interpair second moments measured as a function of temperature in dispersions of the lamellar phases are summarized in Fig. 7.

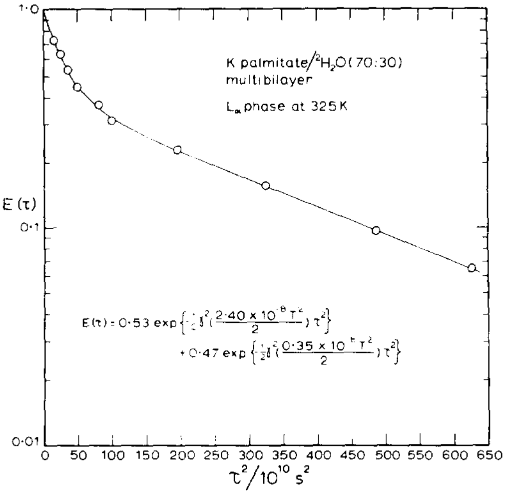

Fig. 5. The dependence of the 90°-τ-90°$_{90°}$ maximum echo amplitude $E(\tau)$ on the square of the pulse spacing τ measured in a multibilayer stack of the lamellar L_α phase of potassium palmitate/^2H_2O. The continuous line represents the best fit of Eqn 3 to the experimental measurements.

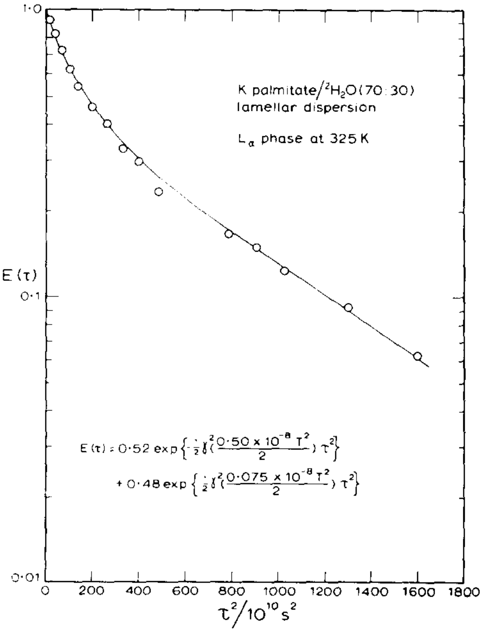

Fig. 6. The dependence of the 90°-τ-90°$_{90°}$ maximum echo amplitude $E(\tau)$ on the square of the pulse spacing τ measured in the L_α phase of a lamellar dispersion of potassium palmitate/^2H_2O. The continuous line represents the best fit of Eqn 3 to the experimental measurements.

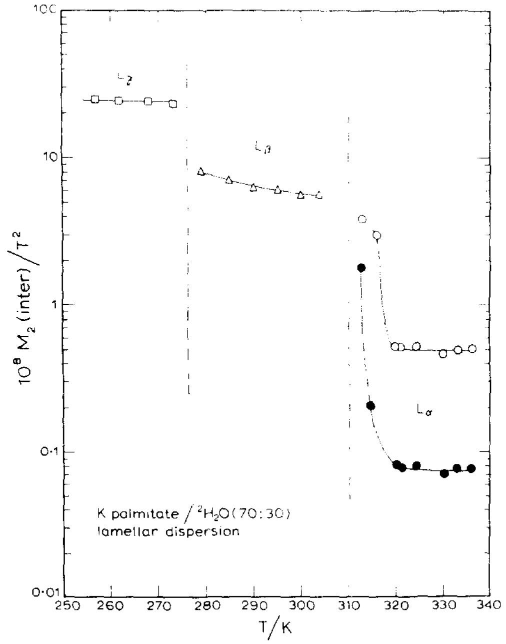

Fig. 7. M_2(inter) measured as a function of temperature in a lamellar dispersion of potassium palmitate/^2H_2O. Type II spin-half pair dipolar echo behaviour is observed in the L_α phase, eqn 3, but type I behaviour, Eqn 2, in the L_β and L_γ phases.

DISCUSSION

^2H and ^{13}C NMR spectroscopy has been used to study segmental motions in lipid alkyl chains. In these experiments the ^2H and ^{13}C spins on different methylene groups, to a good approximation, behave independently. In contrast, the neighbouring methylene proton pairs are strongly coupled so that the NMR experiment measures the behaviour of the entire proton spin system. The value of M_2(inter) obtained from the echo envelope decay is therefore an average over all the proton pairs.

Lamellar phases

For a dispersion of the L_γ phase [18]

$$M_2(\text{inter}) = \tfrac{3}{8}\,\frac{\gamma^2\hbar^2}{N}\sum_{i\neq j} r_{ij}^{-6} \qquad (4)$$

where the summation is carried over protons in N different pairs as for powdered samples of crystalline hydrates. The intramolecular dipolar interactions are calculated to contribute $11.6 \cdot 10^{-8}\ T^2$ to M_2(inter), hence from Fig. 7, we find that the intermolecular contribution is $12.4 \cdot 10^{-8}\ T^2$. The sudden reduction in M_2(inter) at 276 °K is associated with the transition from the low temperature L_γ to the L_β phase. In the latter phase, the chains undergo rapid reorientation about their long axes; this motion averages the intramolecular interpair dipolar interactions along the molecular axes and reduces their contribution to M_2 (inter) to $4.4 \cdot 10^{-8}$ T^2. The residual intermolecular contribution is, therefore $1.7 \cdot 10^{-8}$ T^2.

In the L_α phase, type II behaviour, Eqn 3, is observed with M'_2(inter) and M''_2(inter) independent of temperature. Rapid lateral molecular diffusion over the lamellar surface averages out the residual intermolecular dipolar interactions. Part of the reduction in the interpair second moment is, therefore, due to the averaging of this interaction. The reduction of the intramolecular contribution from $4.4 \cdot 10^{-8}$ to $0.5 \cdot 10^{-8}$ T^2 must, therefore, be due to the onset of intramolecular segmental motions. The interpair second moment is now given by

$$M_2(\text{inter}) = \tfrac{3}{8}\,\frac{\gamma^2\hbar^2 S^2}{N}\,\Sigma_{i\neq j}\left(\left\langle\frac{3\cos^2\phi_{ij}-1}{r_{ij}^3}\right\rangle_{\text{av}}\right)^2 \qquad (5)$$

where S is the order parameter of the long chain axis relative to the bilayer normal (it should not be confused with the segmental order parameters obtained from ^{2}H NMR measurements) and ϕ_{ij} is the orientation of the interproton vector r_{ij} relative to this axis. The double summation is restricted to protons in N different pairs on the same molecule. $\langle(3\cos^2\phi_{ij}-1)/r_{ij}^3\rangle_{\text{av}}$ implies an average over the internal segmental motions of the molecule; the details of the averaging process are, therefore, a function of the location of the methylene proton pairs in the chain. Moreover, only those motions with frequencies above a threshold value of $1 \cdot 10^3$–$1 \cdot 10^4$ s^{-1} provide effective averaging mechanisms for the dipolar interactions. Consequently, the temperature independence of M'_2(inter) and M''_2(inter) indicates that all such segmental motions have been excited.

The observation of type II behaviour implies that the methylene proton pairs can be divided into two distinct spin subsystems. Indeed, this empirical division is also indicated by the shape of the free induction decay signal and the non-exponential Zeeman ($T_{1\rho}$) and dipolar ($T_{1\text{D}}$) spin-lattice relaxation in the L_α phase of potassium laurate [1, 2]. This observation of two spin subsystem behaviour requires that (a) the dipolar interactions within them be described by different Hamiltonians, and (b) the interaction between these two systems must be considerably weaker than the interactions within either of them.

^{2}H NMR measurements in the L_α phases of potassium laurate/water [11], sodium octanoate/decanol/water [8, 9] and dipalmitoyl lecithin/water [10] mixtures, show that in all cases the segmental order parameters are essentially constant for the outer half of the chains, but decrease rapidly for the inner half. It is, therefore, not surprising that in the L_α phases of sodium octanoate/octanol/^2H_2O [17], potassium laurate/^2H_2O and potassium palmitate/^2H_2O, the boundary between the two proton subsystems is located at the centre of the chain. Although in the outer half of the chain the "flip-flop" terms of the dipolar Hamiltonian are energy conserving, this is no longer true for the inner half where the adjacent methylene proton pairs have differing order parameters. This is because the intrapair dipolar interactions are proportional to the segmental order parameters [15]. Thus the "flip-flop" terms are expected to be partially quenched and the dipolar interactions are operationally different for the inner and outer sections of the chains.

To explain the above behaviour it is necessary to postulate the existence of a localized $g^\pm$ defect at a C-C segment which "bends" the chain and divides it into two motionally distinct sections. Thus, M'_2(inter) and M''_2(inter) are determined, respectively, by the dynamic fluctuations within the outer and inner sections of the chain. The ratio of the coefficients A : B = 1 : 1 is temperature independent and indicates that this motional discontinuity is localized at the C_9-C_{10} segment. The ratio 1 : 7 of M''_2(inter) : M'_2(inter) indicates that the inner section of the chain is more dynamically disordered than the outer one.

Further evidence for the "bent" chain hypothesis is furnished by ^{13}C spin-lattice relaxation rate measurements, in vesicles of dipalmitoyl lecithin [7, 20], which also show a discontinuity in the kinetics of the segmental motions at again approximately the centre of the chain.

It should be noted that the "bent" chain model proposed here to rationalize the NMR measurements differs from that earlier proposed by McFarland and McConnell [24] to explain their ESR measurements, in that the outer sections of the chains are parallel to the lamellar plane normal and not tilted with respect to it. Note, however, that the ^1H dipolar interactions are sensitive to lower frequency motions than ESR hyperfine interactions and, moreover, we do not necessarily expect the motional averaging processes to be the same in the NMR and ESR spin-labelling experiments.

Hexagonal phase

The type I spin-half pair dipolar echo behaviour observed in the hexagonal phase indicates a markedly different internal dynamic disorder from that in the L_α lamellar phase. This result implies the presence of an effectively continuous flexibility gradient along the whole length of the alkyl chains. The magnitude of M_2(inter) may be compared with the values measured in the L_α phase. Again, M_2(inter) is solely determined by intramolecular interactions and given by Eqn 5, but in this case its magnitude is reduced further by a factor of 4 due to the translational diffusion over the cylindrical surface. Its value is thus comparable with that of M''_2(inter) for the lamellar phase and indicates that the flexibility of the chain in the hexagonal phase is similar to that of the inner section of the chains in the bilayers. In the hexagonal phase, the long chain axes will be, on average, straight and normal to the cylinder axis.

Intramolecular disorder and its relation to mesophase structure

These measurements show that while in the lamellar L_β and L_γ (gel) phases the alkyl chains are rigid and in their all-trans configuration, i.e. perfectly ordered, in the lamellar L_α and the hexagonal phases they are flexible and disordered. An important result is that the internal disorder is distinctly different for the L_α and hexagonal phases. While in the lamellar L_α phase the chain axes are "bent", in the hexagonal phase they are "straight". Thus, as we have previously shown in the case of thermotropic liquid crystals [16], the disorder is a property of the supramolecular organization of the system and not an intrinsic property of the individual molecule. The characteristic intramolecular disorder in these two phases must be determined by spatial arrangement of the amphiphiles. While the planar arrangement of the molecules in the fluid lamellar L_α phase permits the propagation of long range cooperative conformational waves in the lamellar plane; these "lattice modes" are quenched in the hexagonal phase as they would be restricted to one dimension, i.e. along the axes of the cylinders. The observed behaviour in the hexagonal phase must therefore be entirely due to localized random thermal fluctuations. On the other hand, in the lamellar L_α phase the coherent lattice modes interfere with these local fluctuations.

The existence of a localized $g^\pm$ defect which bends the chain at a central C-C segment in all the lamellar L_α phases we have investigated, could explain why *cis*-ethylenic bonds are frequently found at the C_9-C_{10} positions in naturally occurring phospholipids [21].

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REFERENCES

1 Charvolin, J. and Rigny, P. (1971) J. Magn. Res. 4, 40–46
2 Charvolin, J. and Rigny, P. (1973) J. Chem. Phys. 58, 3999–4008
3 Wennerstrom, H. (1973) Chem. Phys. Lett. 18, 41–44
4 Bloom, M., Burnell, E. E., Valic, M. I. and Weeks, G. (1975) Chem. Phys. Lipids 14, 107–112
5 Seiter, C. H. A. and Chan, S. I. (1973) J. Am. Chem. Soc. 95, 7541–7553
6 Urbina, J. and Waugh, J. S. (1974) Proc. Natl. Acad. Sci. U.S. 71, 5062–5067
7 Levine, Y. K., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1972) Biochemistry 11, 1416–1421
8 Seelig, J. and Niederberger, W. (1974) J. Am. Chem. Soc. 96, 2096–2072
9 Seelig, J. and Niederberger, W. (1974) Biochemistry 13, 1585–1592
10 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845
11 Charvolin, J., Manneville, P. and Deloche, B. (1973) Chem. Phys. Lett. 23, 345–348
12 Boden, N. and Mortimer, M. (1973) Chem. Phys. Lett. 21, 58–62
13 Boden, N., Levine, Y. K. and Squires, R. T. (1974) Chem. Phys. Lett. 28, 523–525
14 Boden, N., Levine, Y. K., Lightowlers, D. and Squires, R. T. (1975) Mol. Phys. 29, 1877–1891
15 Boden, N., Levine, Y. K., Lightowlers, D. and Squires, R. T. (1975) Chem. Phys. Lett. 31, 511–515
16 Boden, N., Levine, Y. K., Lightowlers, D. and Squires, R. T. (1975) Chem. Phys. Lett. 34, 63–69
17 Boden, N., Jackson, P., Levine, Y. K. and Ward, A. J. I. (1975) Chem. Phys. Lett., in the press
18 Boden, N., Levine, Y. K. and Squires, R. T. (1975) J. Chem. Phys., in the press
19 de Vries, J. J. and Berendsen, H. J. C. (1969) Nature 221, 1139–1140
20 Levine, Y. K., Partington, P., Roberts, G. C. K., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1972) FEBS Lett. 23, 203–207
21 van Deenen, L. L. M. (1965) Prog. Chem. Fats Lipids 8, 1–27
22 Luzzati, V., Mustacchi, H., Skoulious, A. and Husson, F. (1960) Acta Cryst. 13, 660–667
23 Husson, F., Mustacchi, H. and Luzzati, V. (1960) Acta Cryst. 13, 668–673
24 McFarland, B. G. and McConnell, H. M. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1274–1277