Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Self-Assembly and Phase Behavior of Short Chain Phosphonic Acid-Water Systems in a Wide Concentration Range Microscopic, NMR, and X-ray Diffraction Studies

G. Klose [a], A. G. Petrov [a b], F. Volke [a], H. W. Meyer [a c], G. Förster [c a d] & W. Rettig [c a d]

[a] Department of Physics, Karl Marx University, 7010, Leipzig, GDR

[b] Medical Department, Institute of Pathology, Friedrich Schiller University, Jena, GDR

[c] Department of Chemistry, Martin Luther University, Halle, GDR

[d] Liquid Crystal Group, Institute of Solid State Physics, Bulgarian Academy of Sciences, Sofia, 1184, Bulgaria

Version of record first published: 13 Dec 2006.

Mol. Cryst. Liq. Cryst., 1982, Vol. 88, pp. 109–126
0026-8941/82/8801-0109/$06.50/0

Printed in the United States of America

Self-Assembly and Phase Behavior of Short Chain Phosphonic Acid-Water Systems in a Wide Concentration Range

Microscopic, NMR, and X-ray Diffraction Studies

G. KLOSE, A. G. PETROV,† F. VOLKE, H. W. MEYER,‡
G. FÖRSTER§ and W. RETTIG§

Department of Physics, Karl Marx University, 7010 Leipzig, GDR

(*Received August 5, 1981; in final form February 16, 1982*)

The phase behavior of *n*-octane and *n*-hexane phosphonic acid-D_2O systems was studied in the concentration range from 0.1 to 90 wt.% and in a temperature range from 10° to 130°C by polarizing and dark field microscopy and by ^1H, ^2D and, ^{31}P NMR. Further, electron microscopy, X-ray diffraction and surface tension measurements were performed. For the first time, a widely spaced lamellar phase was observed over large concentration and temperature ranges in an essentially two-component system. By using the sonication or injection methods, unilamellar liposomes were obtained below 1 wt.%. At high temperatures, the clouding phenomenon was observed. The importance of hydrogen bond formation between polar groups for building up stable lamellae even at high dilution is stressed. The results are discussed with respect to some existing points of view concerning the possibility of lamellar structure formation from single short chain amphiphilic compounds including lipids.

INTRODUCTION

The knowledge that alkane phosphonic acids have surface active properties is relatively new.[1] No studies of their lyotropic mesomorphism have been published up to now. Generally speaking, in water they be-

† Permanent address: Liquid Crystal Group, Institute of Solid State Physics, Bulgarian Academy of Sciences, Sofia 1184 Bulgaria
‡ Medical Department, Institute of Pathology, Friedrich Schiller University, Jena, GDR
§ Department of Chemistry, Martin Luther University, Halle, GDR

have as typical swelling amphiphiles, spontaneously assembling in a broad concentration range into a large variety of lamellar structures which are quite atypical of single chain lipids. The first important observation was the existence of a widely spaced lamellar phase (called type B in the classification of Ekwall[2]) down to very low surfactant concentrations. (According to recent investigations, the B phase is simply a modification of the basic lamellar type D[3,4]). Structures of this type have only been reported up to now as being formed in some ternary amphiphilic-alcohol-water systems.[2]

Similarly, the formation of liposomes is in general observed in two-chain lipids such as lecithins.[5,6] Some wholly synthetic two-chain analogues such as dialkylphosphates[7,8] or dialkylammonium halides[9-11] (and references cited in Ref. 11) have also been successfully employed for this purpose.

There exists also an extensive theoretical treatment by Israelachvili *et al.*[12,13] of the self-assembly phenomenon according to which single chain lipids can only form micelles and rod-like structures in the low concentration limit. The main theoretical result is that these structures are entropically favored. This conclusion is even stronger for the case of short-chain lipids.

However, liposomes from single chain lipids have also been successfully formed, first by use of unsaturated acids[14,15] (the so-called "ufasomes") and then also from saturated fatty acids by titration at a proper pH and at temperatures higher than room temperature.[16]

In the case of *n*-octane phosphonic acid (OPA) and *n*-hexane phosphonic acid (HPA)-water systems as studied by us, giant multilamellar liposomes are spontaneously formed at concentrations $\lesssim 1$ wt.% and at room temperature. This crude dispersion can easily be converted into a dispersion of small unilamellar vesicles by means of sonication or the injection method.

All these results pose a difficult problem with respect to the general validity of the theory of Israelachvili *et al.*[12] One possible answer to this problem is that the importance of the entropic factor is over-estimated so that the theory has a limited validity. As will be seen below there is also another possible answer concerning the special molecular properties of the head group of our compounds and the resulting effective molecular shape.

MATERIALS AND METHODS

(a) Chemicals.

Phosphonic acids of the general formula $H_3C(CH_2)_n P(O)(OH)_2$ with

$n = 5$ (HPA) and $n = 7$ (OPA) were synthesized by Dr. Grossmann (Department of Chemistry, Technical University, Dresden) and used as received. Impurities (octane and hexane, respectively) of 3 wt.% at most must be taken into account. D_2O (Isocommerz, Berlin, 99.5%) was employed in the optical, electron-microscopic, and NMR investigations to ensure identical conditions. For the other studies samples with H_2O were prepared.

(b) Optical studies.

A small amount of the sample substance at a given concentration was placed on a precleaned slide, carefully covered by the coverslip, which was pressed in order to ensure that the whole area below it was covered with the substance, and then sealed with glue. A polarizing microscope (ERGAVAL, Zeiss, Jena) with a heating–cooling stage was used for polarizing microscopy studies. The change of the phase state with changing temperature was continuously studied by recording the integral intensity of the transmitted light through the sample placed between crossed polarizers. Details of this method will be published elsewhere.[17]

Low concentrations were studied by the dark field method at room temperature using a microscope with a xenon arc lamp (NU-2, Zeiss, Jena). The following OPA concentrations were prepared (wt.%): 0.2, 0.45, 1.2, 2.4, 4.1, 7.6, 16.5, 31.2, 65.0, and 82.5. Anhydrous OPA was also studied.

(c) Electron microscopy.

The surface of the anhydrous substance could be examined by the replica technique (Pt + C) using a freeze-etching device without cooling and fracturing. Samples containing water, including dispersions of vesicles (see below) were studied by the freeze-etching method, using a BA 360 M machine (Balzers AG). The vesicle dispersions were equilibrated with 50% glycerol for 10 min and then frozen by means of precooled F22. The replicas were cleaned by rinsing with alcohol and distilled water. For the examination of the replicas a JEM 100B electron microscope (JEOLCO, Tokyo) was used.

(d) NMR-studies.

For ^2D and ^{31}P NMR measurements sealed tubes with a diameter of 7 mm containing the substance were used. For ^2D and ^{31}P NMR they were placed in 10 mm sample tubes with the lock compound. ^2D and ^{31}P spectra were taken at 6.50 and 36.44 MHz, respectively, in the FT regime (HX-90, Bruker). The quadrupole splittings were obtained from

the ^2D spectra by a procedure already described.[18] High resolution ^1H NMR measurements were made at 100 MHz in the CW regime (HA-100, Varian) using 5 mm sealed sample tubes which, besides the substance, also contained a capillary containing the lock compound.

The proton-interpair second moment M_2 (inter) was measured at 32 MHz (BKR-322 S, Bruker) using a $90°$-τ-$90°_{90}$ pulse sequence. The decrease of the maximum-echo amplitude after the second pulse with increasing pulse-spacing time yields the proton-interpair second moment.[19,20]

(e) X-ray diffraction.

These studies were carried out with a HZG3 automatic (step scan) powder diffractometer (VEB Präzisionsmechanik, Freiberg) using a transmission technique.[21] Ni-filtered CuK_α radiation was taken from a TuR M62 generator (VEB Transformatoren- und Röntgenwerk, Dresden). The mixtures were prepared by sealing the various ratios of compound and water in ampoules with an inner constriction. They were centrifugated several times. Finally the specimens were put in thin-walled glass capillaries.

(f) Clouding phenomenon.

This was visually observed using sealed samples and an apparatus for the determination of melting points.

(g) Surface tension.

This was measured by means of the roughened Wilhelmy platinum plate attached to a Cahn-RC-electrobalance. This device allows the reading of surface tension as a function of time.

A cylindrical trough of teflon contains the aqueous solution of the surfactant. The trough is enclosed in a box.

(h) Vesicle preparation.

Two methods for preparing vesicles were employed: sonication of a crude dispersion of the amphiphilic compound for 30 min by a home-made bath-type sonicator working at a frequency of 800 kHz[22] and the injection method of Kremer *et al.*[23] using a solution of the amphiphilic compound (25 mg) in C_2H_5OH (2 ml) which was injected into H_2O (20 ml) at a rate of 10 ml/h (LINEOMAT, VEB Mechanische Geräte, Karl-Marx-Stadt). Both methods are known to produce unilamellar vesicles in the case of lecithin.

RESULTS

(a) Optical investigations.

The melting point of anhydrous OPA was found to be 100°C, with no thermotropic mesomorphism.

Typical textural patterns obtained from some OPA/D_2O samples between crossed polarizers are shown in Figure 1. Comparing the textural appearances of the samples of 82.5 (Figure 1, a) and 65 wt.% (Figures 1, b and c) with the photographs of Ekwall,[2] we see that at those concentrations the lamellar D phase is unambiguously present. The opaque phase observed in the sample of 32 wt.% might be identified as a phase of type C if one judges the situation from the optical pattern (Figure 1, d) and compares it with Figure 27, c of Ekwall.[2] The sample of 16.5 wt.%, visually semi-liquid and transparent, displayed a low contrast texture (Figure 1, f) and might be identified as a phase[2] of type B. Below 16.5 wt.%, the birefringence of this phase is so low that polarizing microscopy is no longer effective. Here we made use of the dark field method. Some results are demonstrated in Figure 2. The B phase in dark field images is displayed as a cloud in which some multilayered giant vesicles are often observed. Most investigators now accept that regions initially labelled C and B are either alternative morphologies of the D phase, or, for many C phase compositions, a mixture of D phase and isotropic micellar solution (L_1).[3,4,24] Below 1 wt.%, only multi-layer liposomes with diameters of some μm were observed freely floating in water and exhibiting Brownian motion (cf. Figure 2, c).

(b) Electron microscopy.

In the frozen state the hydrophobic interior of a bilayer is the region of least resistance for fracture and therefore preferred. By freeze-fracturing, different structures could be observed, as demonstrated in Figure 3, but altogether they are characteristic of a bilayer arrangement. Water-free OPA (Figure 3, a) crystallizes in the form of trigonal pyramids. The preparations with 50 wt.% and 25 wt.% OPA reveal a multilamellar structure (Figures 3, b and c).

In the preparation with 6 wt.% OPA, there is a change from a multilamellar to a single lamellar arrangement with large interlamellar distances (Figure 3, d).

At OPA concentrations lower than 1 wt.%, multilamellar vesicles are revealed quite well. The lamellae are widely separated by water. By the injection method, small vesicles with diameters of about 75 nm

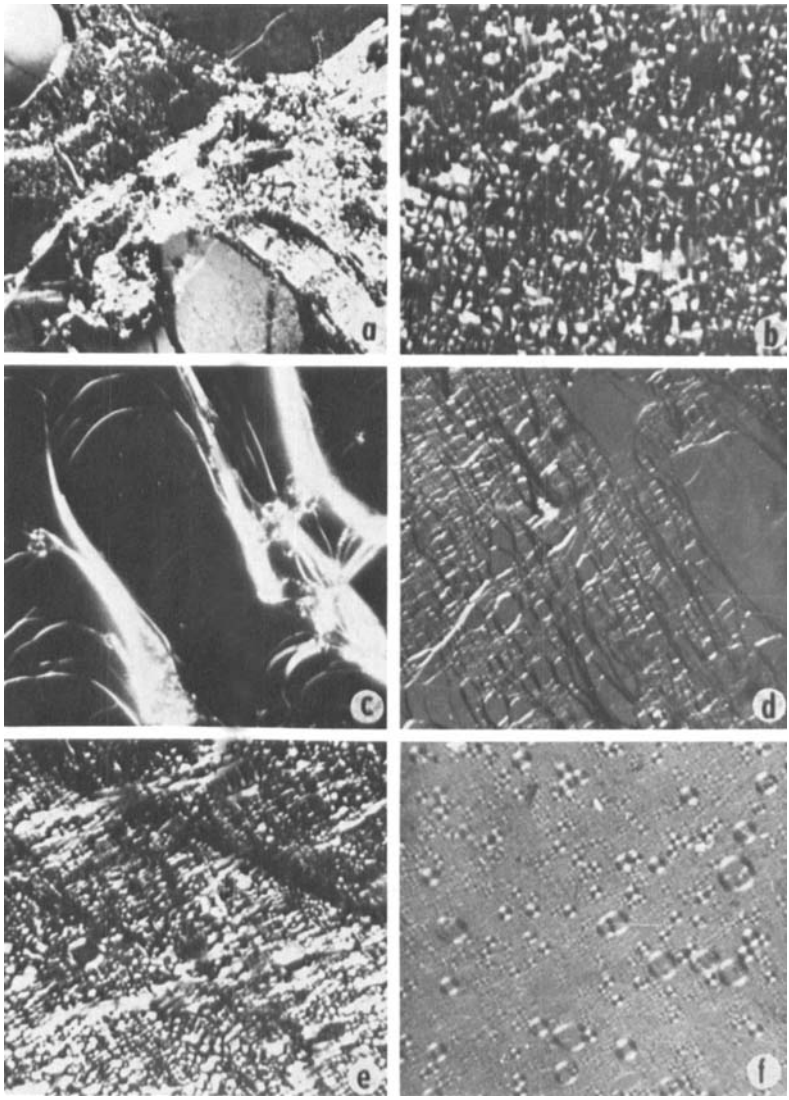

FIGURE 1 Textural patterns of OPA-water systems at 22°C between crossed polarizers. Magnification 250×. (a) 82.5 wt.%, solid and D phases; (b) 65 wt.%, D phase, focalconic texture, (*cf.* Ref 2); (c) 65 wt.%, D phase, planar texture, (*cf.* Ref. 2); (d) 31.2 wt.%, phase C, streaks, (*cf.* Ref. 2); (e) 31 wt.%, phase C and D; (f) 16.5 wt.%, lamellar phase type B (low contrast).

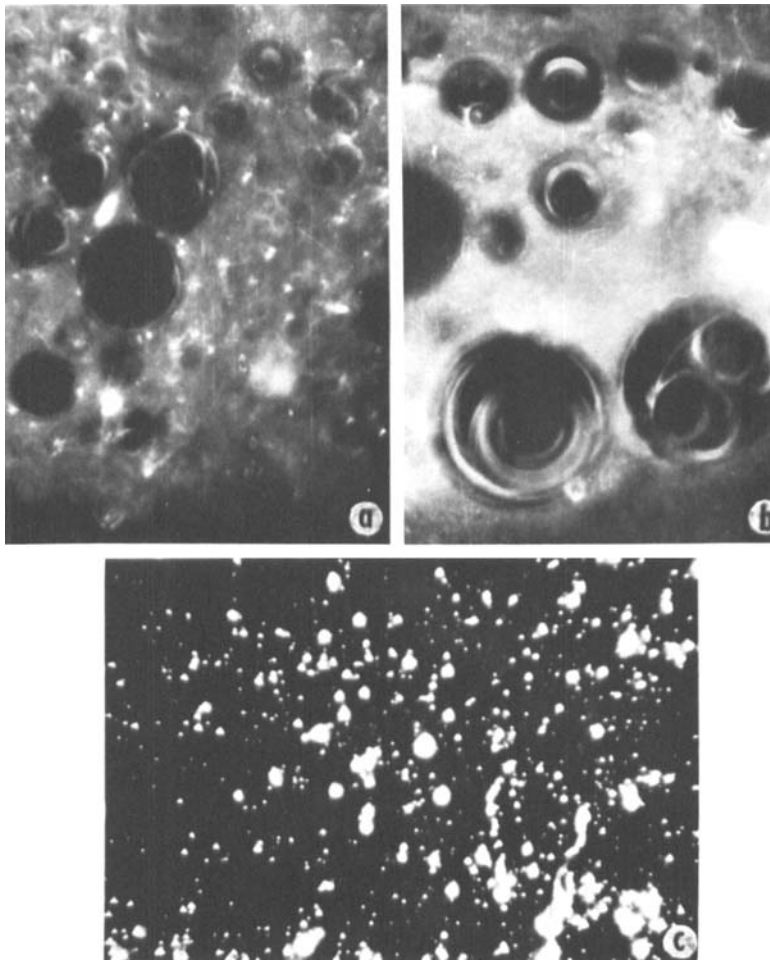

FIGURE 2 Dark field appearance of OPA-water systems at 25°C. Magnification 1500×. (a) 2.4 wt.%, lamellar B phase; (b) 1.2 wt.%, spherulites in B phase; (c) 0.45 wt.%, vesicles. Black areas inside the giant vesicles and liposomes in (a) and (b) correspond to the absence of a continuous B-phase, which is observed outside only.

(with a narrow diameter distribution) can be obtained. The sonication method, however, gives a broad spectrum of spherical vesicles with diameters from 50 to 1000 nm as demonstrated in Figures 3, e and f.

The fine textures observed on the fracture faces (Figure 3) indicate that the cooling rate in the freezing procedure is not sufficiently fast to prevent partial transformation into the crystalline state. By fast

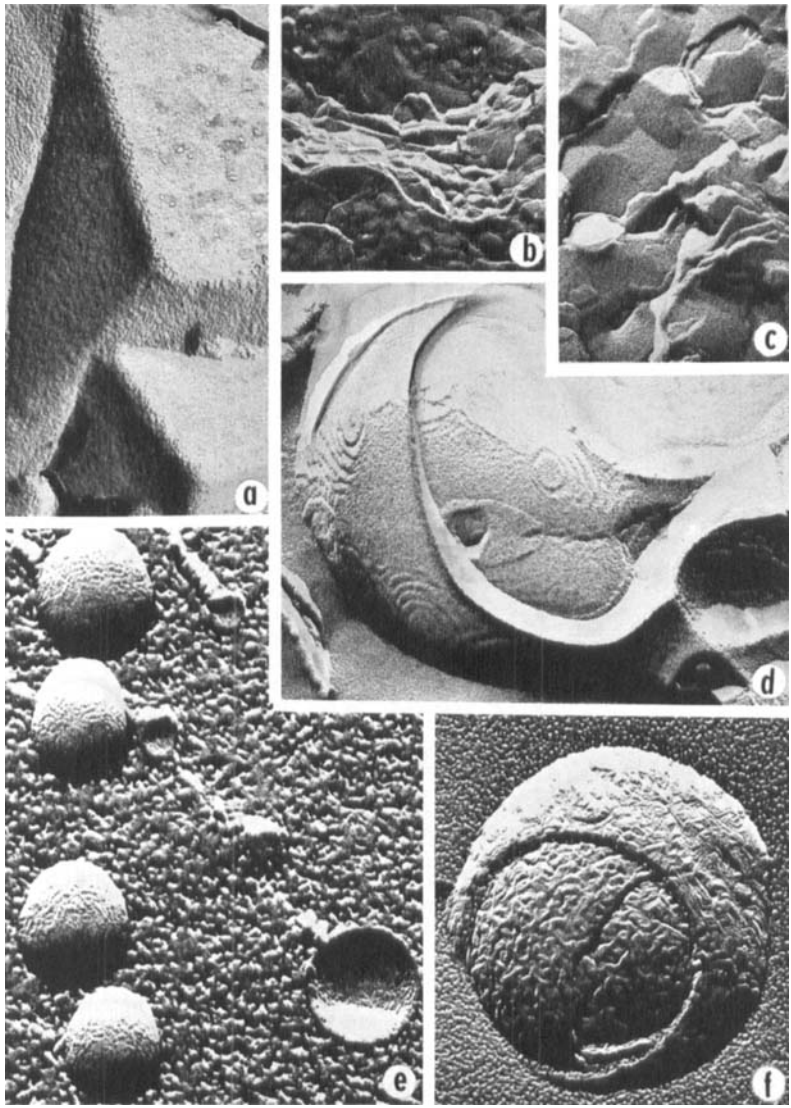

FIGURE 3 Freeze-fracture appearance of OPA-D_2O systems. Magnification: a, b: ×50,000; c, d, e: ×100,000, f: ×60,000. Direction of shadowing from bottom to top. (a) Surface replica of water free OPA; (b) 50 wt.% OPA, multilamellar structure; (c) 25 wt.% OPA, multilamellar structure; (d) 6 wt.% OPA, single lamellar structures; fracture face of the lamellae with multistep areas forming a circular line pattern; (e) 0.5 wt.%, small vesicles of different sizes (prepared by sonication); (f) 0.5 wt.% OPA, large vesicle, with enclosed vesicles (prepared by sonication).

quenching of the samples from room temperature by means of a sandwich technique, a smooth textureless surface for the vesicles was found.[25]

(c) NMR Investigations.

The ^2D spectra of D_2O (Figure 4, a) in samples with concentrations equal to or higher than 30 wt.% OPA exhibit line shapes which are characteristic of anisotropic mesophases.[26-28] The spectra of samples with concentrations equal to or lower than 25 wt.% OPA do not show any indication of a quadrupole splitting.

The quadrupole splittings obtained from the spectra are represented as a function of temperature in Figure 5. The vanishing of the quadrupole splittings with rising temperature gives the temperatures of the transition into the isotropic phase. In all samples, complicated spectra were observed, with two quadrupole splittings or one quadrupole splitting and one isotropic line, in the small temperature range of a few degrees represented in Figure 5 by broken lines. Apparently two phases co-exist in these transition ranges to the isotropic state. Visually one observes a phase separation into two transparent phases.

The drastic decrease of the quadrupole splitting as the temperature decreases from 40 to 35°C in a 90 wt.% OPA sample (cf. Figure 5) probably indicates a partial crystallization of OPA.

An interpretation of the observed quadrupole splittings is difficult because they are the weighted averages of the effective quadrupole splittings of the water molecules in the different interaction sites present in the system and additionally of those of the acidic deuterons. A

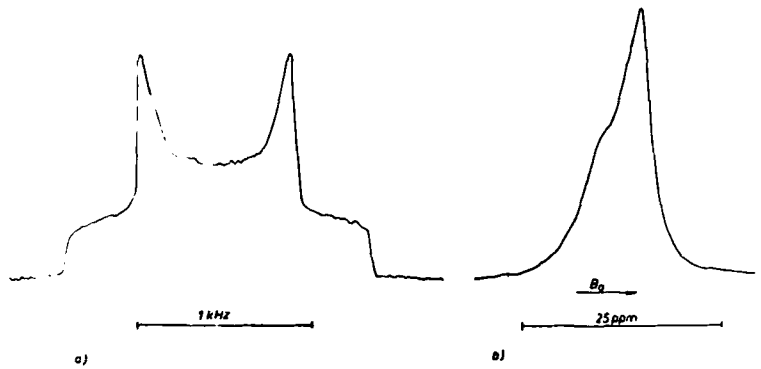

FIGURE 4 NMR spectra of OPA-D_2O systems (80 wt.%) at 20°C. (a) ^2D spectrum of D_2O; (b) ^{31}P spectrum of OPA with strong proton noise decoupling.

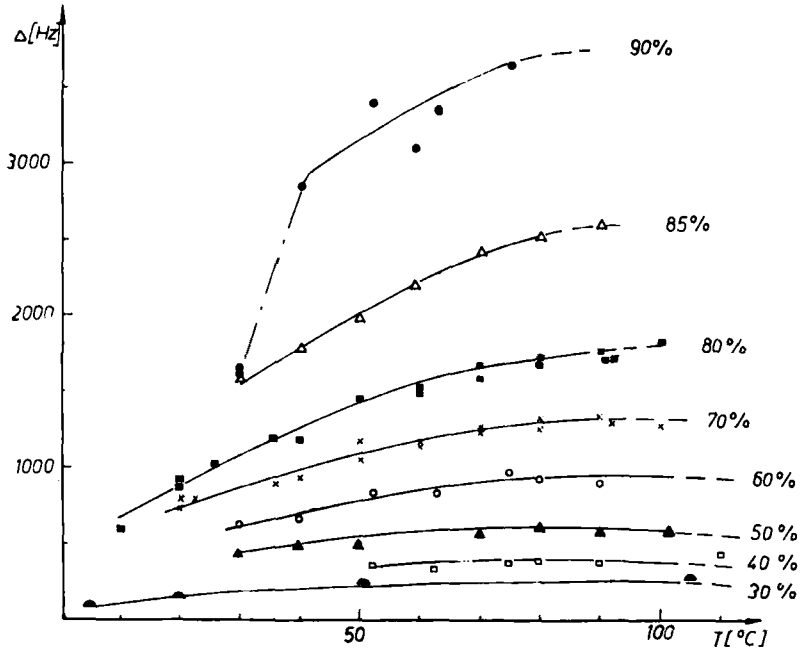

FIGURE 5 Quadrupole splittings measured for OPA/D_2O systems at different concentrations as a function of temperature.

change of splitting can be the result of change in the populations of the various sites or change in the effective quadrupole splitting in the sites.

In a model which considers, besides the "bound" and "free" water, the acidic deuterons, the observed splitting Δ is given in the case of $X_A \ll X_{D_2O}$ by

$$\Delta = \frac{X_A}{X_{D_2O}} |(n\Delta_{D_2O} + k\Delta_{AD})|$$

provided that the "free" water molecules as well as the dissociated acidic deuterons exhibit zero splittings and that the exchange processes are fast. n is the average number of water molecules bound to the amphiphile and Δ_{D_2O} and Δ_{AD} are the effective quadrupole splittings of bound water molecules and the non-dissociated acidic deuterons, respectively. k describes the dissociation states of the amphiphile. It is 1 in the case of no dissociation and 0.5 if one acidic deuteron is dissociated on average. X_A/X_{D_2O} is the molar ratio of the amphiphile and water.

A plot of Δ *vs.* X_A/X_{D_2O} should give a straight line through the origin

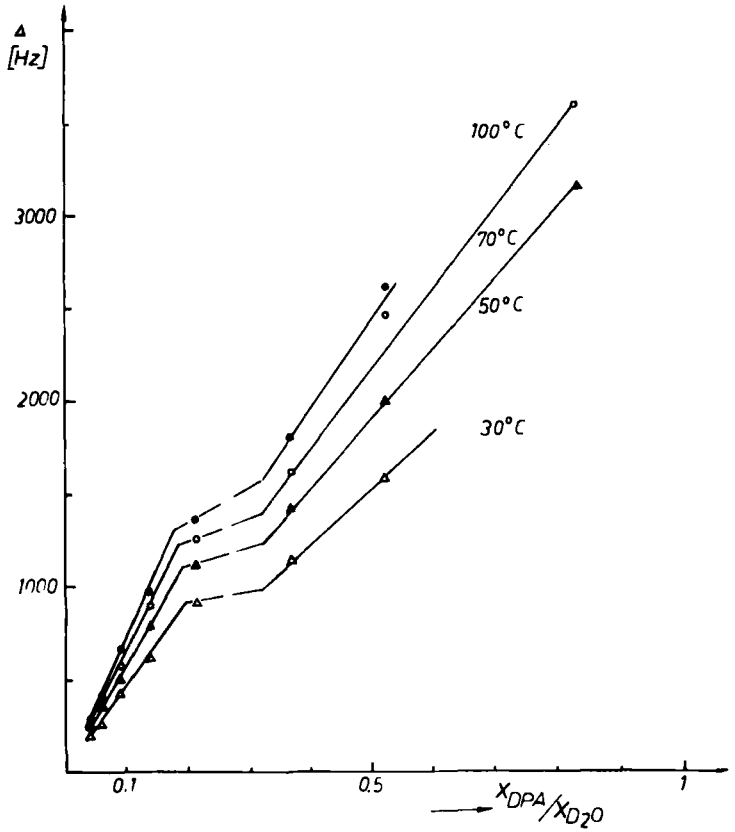

FIGURE 6 Quadrupole splittings measured for OPA/D_2O systems at different temperatures as a function of the molar ratio X_{OPA}/X_{D_2O}.

with a slope equal to $n\Delta_{D_2O} + k\Delta_{AD}$. Figure 6 shows such plots for OPA/D_2O systems. Obviously there are two regions in which the plots give straight lines, but with different slopes. Above $X_{OPA}/X_{D_2O} \approx 0.35$ the slopes are smaller than below ≈0.2.

In the framework of our model, these results can be interpreted as being due to an increase in the number of water molecules bound to OPA or/and to a change of the dissociation state of the amphiphile when the molar ratio X_{OPA}/X_{D_2O} is decreased from 0.35 to 0.2. Clarification of this needs further experiments (e.g. ^{17}O NMR of $H_2\,^{17}O$).

At low concentrations, where no quadrupole splitting of D_2O is observable, high-resolution PMR was applied to the determination of the temperature of the transition from the isotropic to the anisotropic

phase with decreasing temperature. The integral intensity of the CH_2 chain protons was used as a parameter indicating this transition. The temperatures at which the intensity dropped to one half of its value for the isotropic phase due to the formation of liquid crystal states with restricted mobility are marked with full points in Figure 7. The water signals also indicate certain transition ranges as shown by the occurrence of two HDO lines. This is indicated by the hatched region in Figure 7.

At room temperature, only at concentrations smaller than about 1 wt.% OPA high-resolution PMR spectra could be observed. Evidently at these low concentrations vesicles are present that are small enough for the static dipole–dipole interactions to be averaged to zero by their fast reorientations. Indeed, this was confirmed by dark field and by electron microscopy.

The ^{31}P spectra obtained with strong proton noise decoupling at concentrations equal to or higher than 15 wt.% OPA exhibit a line shape (Figure 4, b) which resembles those observed for phospholipids

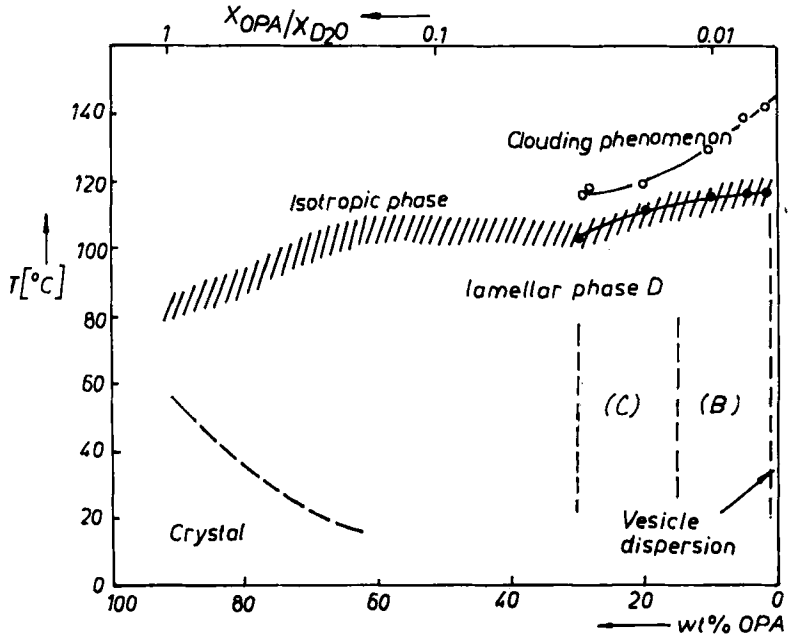

FIGURE 7 Crude phase diagram of OPA/D_2O mixtures. Hatched region indicates liquid crystal to isotropic transition range according to ^{2}D-NMR and PMR (two phase region). Changes in optical appearances indicate alternative morphologies of the D phase for regions labelled (C) and (B).

in the fluid lamellar phase.[29] The asymmetry of the lines disappears at the transitions to the isotropic phase. Also, no asymmetry of the lines is present in samples with concentrations lower than 1 wt.%.

The line asymmetry is caused by the anisotropies of the chemical ^{31}P screening which is not averaged to zero in the anisotropic phases except at low concentrations, but is only reduced to a great extent (observed anisotropy, only about 4 ppm) by motional processes and by exchange of the acidic deuterons.[27] By analogy with phospholipids and according to theoretical calculations,[30] it is probable that the largest and the smallest principal screening value are perpendicular and parallel respectively to the molecular symmetry axis (hydrocarbon chain). The asymmetric line shapes observed for the anisotropic phases are in agreement with lamellar structures.

On storing the samples for several hours at 100°C, a second ^{31}P line (under proton noise decoupling conditions) appears; this results from a decomposition product.

Water free OPA yields a proton-interpair second moment, M_2(inter), of $3.3 \cdot 10^{-8}T^2$. It is interesting that the same value was obtained for the water free mono-sodium salt of dipalmitoylphosphatic acid.[31] M_2(inter) is reduced compared with the value for the rigid *all-trans* octane chain of about $8 \cdot 10^{-8}T^2$ for a powder or $13 \cdot 10^{-8}T^2$ for a perfectly ordered octane chain oriented parallel to the external magnetic field.[32]

For the 50 wt.% OPA/D_2O system, we found a smaller M_2(inter) = $0.16 \cdot 10^{-8}T^2$ at 290 K which continuously decreases to $0.12 \cdot 10^{-8}T^2$ at 335 K. This means that dipolar interactions between neighboring methylene groups are averaged out to a large extent by fast *trans-gauche* transitions.[20] For phospholipids in the fluid liquid crystal state, C_{16} chains were found[33] to have an average M_2(inter) = $0.33 \cdot 10^{-8}T^2$. A further reduction of this value is only possible either by increasing the average distance between the chains or by additional motions, e.g., in the case of a transformation into the hexagonal phase.[34] In the case of the hexagonal phase, M_2(inter) should be smaller than $0.1 \cdot 10^{-8}T^2$. Therefore, the M_2(inter) studies also lead to the conclusion that the 50 wt.% OPA dispersion exists in a D state.

(d) X-ray diffraction studies.

In the water free state, X-ray diffraction patterns characterize OPA as a three-dimensional crystal. Up to high angles ($0.5° \leqslant 2\theta \leqslant 48°$), there exist many sharp reflections, and accurate values for unit cell parameters are available only after indexing all experimental spacings. But the long spacings (d_1 = 1.841 nm, d_2 = 0.904 nm, d_3 = 0.598 nm) can be

interpreted as integers of the repeat distance d_{001} of a lamellar structure which yields an approximate value of 1.8 nm for the lamellar thickness. As seen from space-filling models, the extended OPA molecule is about 1.5 nm long, with a length of the hydrocarbon chain of 1.2 nm. Assuming an interdigitated arrangement of the molecules in the bilayer, the calculated thickness of (1.2 + 2·0.3) nm = 1.8 nm fits the experimentally obtained value.

The assumption of tilted chains yields a tilt angle of 53° between the director of the lamellae and the chain axis. Such a figure is very unlikely.

The diffraction pattern for a 50 wt.% OPA sample characterizes the physical state of the hydrocarbon chains (short spacings), as well as the superlattice structure (long spacings). A broad reflection corresponding to a spacing of about 0.046 nm indicates that the hydrocarbon chains are in a liquid-like state in accordance with the NMR results. The low angle X-ray diffraction data show that a one-dimensional lamellar lattice exists. Long spacings in the ratios of 1:1, 1:2, 1:3 are observed (d_1 = 4.73 nm, d_2 = 2.34 nm, d_3 = 1.66 nm), from which an average repeat distance $d_L \approx 4.8$ nm is calculated. This value includes the thickness of the bilayer as well as the water layer. The lamellar structure of the 50 wt.% OPA sample apparently has similarities to the well-known lamellar phase described in detail by Gulik-Krzywicky *et al.*[35] These phases are characterized by lipid bilayers with molten chains and wide water layers between them.

(e) Surface tension measurements.

The critical micelle concentration (CMC) obtained from the plot of surface tension against log concentration amounts to 10^{-2} mol/l at 22°C. This value is in the order of magnitude which is characteristic of non-ionic surfactants. For instance, for dimethyloctylphosphinoxide, the CMC was found to be $4 \cdot 10^{-2}$ mol/l at 30°C.[36] The surface tension decreases from 72.8 N/m (pure water) to the very small value of 19 mN/m at the CMC. This fact indicates a strong interaction between the phosphonic acid groups and water.

(f) Clouding phenomenon.

The existence of a clouding point at high temperatures demonstrates once again the striking similarity between our compounds and non-ionic detergents (Figure 7).

DISCUSSION

Comparing the phase behavior of short chain phosphonic acids with that of soaps (see e.g. Ref. 2), the former seems rather strange. The tendency to form lamellar structures found experimentally by different methods can hardly be understood by considering only the high cone-like asymmetry of the shape of the single molecules which consequently should preferably pack into micelles at low concentrations.[12] The transition temperature to the isotropic state increases with decreasing concentration as in non-ionic surfactants having low melting points, e.g. mono-glycerides.[2,4]

We think that all these facts can be explained by assuming that strong hydrogen bonds exist between the bulky head groups, in addition to the hydrophobic interactions. As a simple model, it can be imagined that in the fluid states, two molecules form a short-lived dynamical aggregate (head to head and with parallel hydrocarbon chains) which is effectively cylindrical. This effective shape needs, of course, a high flexibility of the chains, which is fulfilled because of their shortness and proved by the second proton-interpair moment and X-ray diffraction results (see above). The hydrocarbon chains of the two layers facing each other might in addition be interdigitated to some extent, as found for the water free compound in the crystalline state. Such interdigitation is known in other systems in the gel state[37,38] and is discussed[2,9,40] and also experimentally verified[38,41] for the liquid crystal phase. The tendency of these aggregates to form lamellar structures is then clear, and all considerations for double-chained lipids[13] can be applied to them. Lawrence[42] (as long ago as 1969) indicated that two OH residues on a head group are sufficient for the D phase to occur and OPA and HPA are further examples of his statement. But other factors such as for example low chain melting points are also important (see below).

With dilution, the amphiphilic molecules increasingly dissociate ($pK_1 = 2.77$, $pK_2 = 7.65$).† If we assume that the electrostatic repulsion between the molecules in one layer is overcompensated by stronger hydrogen bonds in respect to undissociated molecules, then the increase in the transition temperature to the isotropic phase with decreasing concentration of the amphiphile can be understood.

The strong electrostatic repulsion between the layers stabilizes the lamellar structures down to extremely low concentrations of the am-

† Measured at 25°C and a concentration lower than 10^{-2} molar.

TABLE I

Regions of transition from liquid crystal to isotropic phase for *n*-hexane phosphonic acid-water systems

Concentration HPA/wt.%	70	50	40	30	20	10	4	2
Transition region/°C	25–29	24–28	22–29	38–47	48–51	49–53	56	50–52

phiphile (1 wt.%). This, together with the presence of two OD groups in the polar head, could be the reason that a widely spaced lamellar phase is possible in our two-component systems.

At concentrations lower than 1 wt.%, the electrostatic repulsion between the bilayers is too weak to be able to stabilize a widely spaced lamellar phase. The result is that curved multilayer aggregates become entropically more favorable than the continuous widely spaced lamellar phase. They can be transformed into stable bilayer liposomes by sonication or other methods.

Entirely the same phase behavior was found for *n*-hexane phosphonic acid-water systems, the only difference being that the region of transition from the liquid crystal phase to the isotropic phase is shifted to lower temperatures—about 60°C (Table I). This can be understood as being due to weaker dispersion interactions between the hydrocarbon chains of HPA in comparison with OPA.

We also studied *n*-dodecyl phosphonic acid over the same temperature and concentration ranges as for the short chain phosphonic acids. In this case liquid crystal phases could not be detected. Apparently the chains are too rigid because of their strong dispersion interactions (high melting points). On the other hand, the disodium salts of HPA and OPA dissolved in water give only isotropic solutions, but those of *n*-decyl, *n*-dodecyl- and of *n*-hexadecyl phosphonic acids again exhibit liquid crystal phases.[43] These facts again emphasize the importance of hydrogen bonding in our phosphonic acid-water systems.

Acknowledgments

The authors thank Dr. Dietze for measuring the p*K* values of OPA. Further, they thank Mrs. Neitzsch for preparing the samples and running the NMR spectra, Mrs. Brandt for technical assistance, and Dr. Dörfler (MLU, Halle) and Dr. Haage (ZIOC, Berlin) for helpful discussions. Thanks are also due to the referee for stimulating criticism and to Prof. Stenius for sending us a preprint of his paper. A. G. Petrov is also indebted to the Karl Marx University for his invitation there and for financial support.

References

1. L. Maier, *Chimia*, **23**, 323 (1969).
2. P. Ekwall, in *Advances in Liquid Crystals* (Academic Press Inc., New York, San Francisco, London) Vol. 1, 1975 p. 1–142.
3. R. Friman, I. Danielson and P. Stenius, *J. Coll. Interface Sci.* (in press).
4. C. J. T. Tiddy, *Phys. Reports*, **57**, 1 (1980).
5. D. A. Tyrrell, T. D. Heath, C. M. Colley and B. E. Ryman, *Biochim. biophys. Acta*, **457**, 259 (1976).
6. A. D. Bangham, *Progr. Biophys. Mol. Biol.*, **18**, 29 (1968).
7. R. A. Mortara, F. H. Quina and H. Chaimerich, *Biochem. biophys. Res. Comm.*, **81**, 1080 (1978).
8. M. Czarniecki and R. Breslow, *J. Am. Chem. Soc.*, **101**, 3675 (1979).
9. T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, **99**, 3860 (1977).
10. K. Kano, A. Romero, B. Djermoni, J. H. Ache and J. H. Fendler, *J. Am. Chem. Soc.*, **101**, 4030 (1979).
11. J. R. Sudhölter, G. B. van de Langkruis and J. B. F. N. Ergberts, *Rec. Royal Netherlands Chem. Soc.*, **99**, 73 (1980).
12. J. N. Israelachvili, J. D. Mitchell and B. W. Ninham, *J. Chem. Soc. Faraday Trans.*, **272**, 1525 (1976).
13. J. N. Israelachvili, D. J. Mitchell and B. Ninham, *Biochim. biophys. Acta*, **470**, 185 (1977).
14. J. M. Gebicki and M. Hicks, *Nature*, **243**, 232 (1973).
15. J. M. Gebicki and M. Hicks, *Chem. Phys. Lipids*, **16**, 142 (1976).
16. W. R. Hargreaves and D. W. Deamer, *Biochemistry*, **17**, 3759 (1978).
17. A. G. Petrov, K. Gawrisch, G. Klose and A. Möps, *Biochim. biophys. Acta* (submitted).
18. K. Gawrisch, *Z. Chem.* (in press).
19. N. Boden and Y. K. Levine, *J. Magn. Reson.*, **30**, 327 (1978).
20. F. Volke, Doctoral Thesis, Leipzig, 1981.
21. G. Förster, G. Brezesinski, E. Gerlach, A. Madicke and H.-D. Dörfler, *Z. phys. Chem.*, (1981) (in press).
22. H. Frischleder, R. Grupe, M. Gründel and H. Winkelmann, *Studia biophysica*, **68**, 195 (1978).
23. J. M. H. Kremer, M. W. J. v.d. Esker, C. Pathmamanoharan and P. H. Wiersema, *Biochemistry*, **16**, 3932 (1977).
24. P. Stenius and P. Ekwall, *Surface Chemistry and Colloids*, (Ed. M. Kerker) *MTP Intern. Rev. Sci., Phys. Chem.*, Ser. 2, Vol. 7, 215 (1975).
25. H. W. Meyer and G. Klose, *Exp. Pathology*, **21**, Heft 2 (1982) (in press).
26. H. Wennerström, G. Lindbloom and B. Lindman, *Chemica Scripta*, **6**, 97 (1979).
27. G. Klose, Sixth School on Biophysics of Membrane Transport, School Proceedings, Poland 1981, Part I, p. 7.
28. N.-O. Person and G. Lindbloom, *J. Phys. Chem.*, **83**, 3015 (1979).
29. K. Arnold, K. Gawrisch and F. Volke, *Studia biophysica*, **75**, 189 (1979).
30. Th. Weller, private communication.
31. F. Volke, private communication.
32. N. Boden, Y. K. Levine, D. Lightowlers and R. T. Squires, *Chem. Phys. Lett.*, **34**, 63 (1975).
33. D. F. Bocian and S. I. Chan, *Ann. Rev. Phys. Chem.*, **29**, 307 (1978).
34. J. Charvolin and A. Tardieu, *Solid State Phys.*, **14**, 209 (1978).
35. T. Gulik-Krzywicki, A. Tardieu and V. Luzzati, *Mol. Cryst. Liq. Cryst.*, **8**, 285 (1969).
36. K. W. Herrmann, I. C. Brushmiller and W. L. Cowchene, *J. Phys. Chem.*, **70**, 2909 (1966).
37. V. Luzzati, *Biological Membranes* (Ed. D. Chapman), Academic Press, London, Vol. 1, p. 71–123 (1968).

38. J. Vincent and A. E. Skoulios, *Acta Cryst.*, **20**, 432 (1966).
39. P. A. Winsor, *Chem. Rev.*, **68**, 2 (1968).
40. H. Kelker and R. Hatz, *Handbook of Liquid Crystals*, Verlag Chemie, Weinheim, Deerfield p. 516–523 (1980).
41. R. M. Hedge, R. K. Thomas, M. Mortimer and J. W. White, *J. Chem. Soc.*, Faraday I, **76**, 236 (1980).
42. A. S. C. Lawrence, *Mol. Cryst. Liq. Cryst.*, **7**, 1 (1969).
43. G. Klose, K. Arnold, K. Gawrisch and F. Volke, *Proc.* Third Liquid Crystal Conference, Budapest, 1979, Vol. 2, p. 787.