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POLYMORPHISM OF LIPIDS
A NOVEL CUBIC PHASE—A CAGE-LIKE NETWORK OF RODS WITH ENCLOSED SPHERICAL MICELLES

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SUMMARY

Among the numerous lipid–water phases a few crystallize in the cubic system and offer interesting crystallographic problems. One of these (space group Ia3d) consists of rods of finite length, joined three by three and forming two three-dimensional networks, mutually interwoven and unconnected. We describe the structure of another widespread cubic phase, space group Pm3n. The structure consists of a three-dimensional network of rods of finite length, all crystallographically equivalent, which meet three by three at one end and four by four at the other and form the bars of a system of cages, each of which encloses a spherical micelle (chlathrate-type structure). The "liquid" hydrocarbons occupy the interior of the rods and spheres, the water fills the interstices. The hydrocarbon–water interface is covered by the polar groups of the lipid molecules.

INTRODUCTION

Until a few years ago the crystallography of lipid–water systems was presented as a shallow subject, involving only lamellar structures. A systematic study undertaken by our group some 15 years ago, did in fact uncover quite a complex polymorphism with a large number of phases displaying a surprising variety of structures (see review in ref. 1). These structures may be sorted in two main classes: (a) lamellar, containing planar elements, which may be indefinite sheets packed in a one-dimensional lattice, ribbons indefinitely long and of finite width packed in two-dimensional lattices or discs of finite dimensions packed in three-dimensional lattices; (b) rod-like, containing either indefinitely long and stiff rods packed in a two-dimensional hexagonal lattice, or identical rods of finite length joined three by three or four by four and organized in a variety of three-dimensional lattices (see DISCUSSION below). In most of the phases the short-range organization of the hydrocarbon and of the water regions is of the liquid type. Besides, two types of structures are observed whenever a topological distinction can be made between the internal and the external volumes of the structure elements, those with the hydrocarbons "inside" and the water "outside" (Type I), and *vice versa* (Type II).

From the crystallographic standpoint one family of phases, crystallizing in the cubic systems, is particularly interesting. One of these, commonly observed in a variety of lipid–water systems in the concentration range intermediate between the lamellar and the hexagonal phases, has been the object of several recent reports[1–4]. The space group is Ia3d; the structure consists of rod-like elements of finite length, linked three by three into two three-dimensional networks, mutually interwoven and unconnected (see Fig. 1 in ref. 4). In some systems the interior of the rods is filled by the hydrocarbon chains, in others by the polar groups[4].

We have reported recently[5] the observation of several other cubic phases. We discuss here the determination of the structure of one of these. This phase was found originally in three-component systems involving sodium caprylate, organic solvents and water[6]. It has since been observed in several two-component lipid–water systems: dodecyl trimethylammonium chloride[7], egg lysolecithin (A. TARDIEU, unpublished), lauryl decaethylene glycol ether (A. TARDIEU AND K. FONTELL, unpublished) and in a variety of three-component systems containing sodium or potassium soaps, water and organic solvents (heptane, decane, *p*-xylene). This phase, like all the cubic phases, is optically isotropic and highly viscous. It easily yields fairly large crystals (to the extent that a rotating sample holder had to be used in order to record satisfactory X-ray powder diagrams), and its X-ray diffraction diagrams contain the 4.5 Å band typical of the "liquid" paraffins[1]. Two features distinguish this phase from the other cubic phases: its position in the phase diagram encompassed by the hexagonal and the micellar phases (thus at a high water content), and the spacings ratios of the small-angle reflexions (see Table I).

We have previously reported[5] the space group of this phase, which was chosen according to the usual principle that the number of missing reflexions attributed to the systematic absences should be a maximum. An inspection of the X-ray diffraction diagrams, obtained with a variety of systems, leads to two possible space groups, $P\bar{4}3n$ and Pm3n (No. 218 and 223 of the International Tables[8]). The space group was since confirmed by single crystal analysis[7].

The subsequent steps in the structure analysis were guided by our previous experience with lipid–water systems. First, we assume that the structure elements (namely the regions occupied by the hydrocarbons or by the polar moiety) occupy special positions of the space group—indeed the shape of the structure elements is likely to be defined by the symmetry of the cell, since both the paraffin and the polar regions lack internal rigidity[1,3]. Second, we adopt the rule that the area per polar group at the paraffin–water interface never decreases as the water content increases, even when phase boundaries are crossed[1,4]. Third, we suppose that the distribution of water and paraffins is of Type I, namely, the paraffins being inside the structure elements (rods and spheres, see below) and water outside, on the grounds that in all the systems described here the order of the sequence in the phases with increasing water concentration—lamellar, cubic Ia3d, hexagonal, cubic Pm3n, micellar—is typical of structures of Type I (refs. 1, 4). Fourth, we assume that the two media —paraffin and water—are continuous through each crystal and are mutually entangled in a three-dimensional network, in keeping with the high viscosity and easy growth of large crystals, two properties characteristic of the cubic phases (see discussion in refs. 3 and 4).

The different sets of special positions of the two space groups may now be discussed (see International Tables[8]).

TABLE I

X-RAY DIFFRACTION DATA

The data were obtained with the system sodium caprylate–*p*-xylene–water. The spacings of the observed reflexions are in perfect agreement with a cubic lattice ($a = 75.0$ Å) and with the indices given in the table. The intensities were measured as discussed in ref. 13. I_{cal} takes the form: $I_{hkl} = m\ [F_1(hkl)\ f_1(s) + F_2(hkl)\ f_2(s)]^2 \exp(-\alpha s^2)$. m is the multiplicity factor, $F_1(hkl)$ is the structure factor of the framework of rods with gaps of length ε' and ε'' at the ends[10], $F_1(hkl) = 1/8 \int_{\varepsilon''/4l}^{1/4-\varepsilon'/4l} A_{hkl}\ (1/4, x, 1/2+x)\ \mathrm{d}x$; $f_1(s)$ is the form factor of a long rod formed by a paraffin cylinder of radius R_{par} surrounded by a polar shell of radius R_{pol}: $f_1(s) = J_1(2\pi R_{\mathrm{pol}}s)/\pi R_{\mathrm{pol}}s - K_1 J_1(2\pi R_{\mathrm{par}}s)/\pi R_{\mathrm{par}}s$. $F_2(hkl)\ f_2(s)$ is the contribution of the spherical particles, formed by a paraffin core surrounded by a polar shell. $F_2(hkl)\ f_2(s) = K\ [\Phi(2\pi R_{\mathrm{pol}}s) - K_2\Phi(2\pi R_{\mathrm{par}}s)]$; $\Phi(x) = 3\ (\sin x - x\cos x)/x^3$; this contribution is $\neq 0$ only for $h + k + l = 2n$. The numerical values of the parameters are the following. The volume concentrations and the partial specific volumes of sodium caprylate, *p*-xylene and water are: 0.41–0.05–0.54:0.908–1.16–0.968 $\mathrm{cm^3 \cdot g^{-1}}$. The radii of the paraffin and polar shells are: $R_{\mathrm{par}} = 9.0$ Å, $R_{\mathrm{pol}} = 10.7$ Å for the rods; $R_{\mathrm{par}} = 14.5$ Å, $R_{\mathrm{pol}} = 15.9$ Å for the spheres. The lengths of the gaps are $\varepsilon' = 2.8$ Å, $\varepsilon'' = 4.9$ Å; the length of each rod is $l = a/\sqrt{8} = 26.5$ Å. The volume ratio of spheres to rods (contained in one unit cells) is $K = 0.20$. The adjustable parameters take the values; $K_1 = 0.82$; $K_2 = 0.69$:$\alpha = 1300$ Å^2. The I_{obs} are normalized assuming ΣI_{obs}:ΣI_{cal}.

hkl	*I*	
	Calc.	*Obs.*
110	100	60
200	390	275
210	1700	1520
211	620	1060
220	20	23
310	28	27
222	2	< 7
320	10	10
321	19	15
400	28	25
410	110	27
411 330	3 1	<10
420	47	31
421	48	64

Positions *a, b, e, f* of P$\bar{4}$3n and positions *a, b, f, i* of Pm3n are discarded as they involve a restriction ($h+k+l = 2n$) clearly incompatible with the observed reflexions (see Table I). Similarly, position *e* of Pm3n is discarded (restriction $h, k, l = 2n$) as well as positions *c* and *d* of both space groups (for these, the reflexion [410] would be absent).

Positions *h* and *g* of both space groups correspond to a system of rods lying along 2-fold axes. Knowing the volume concentration and the parameter of the unit cell, the radius of the rods can be determined once their length is chosen. It turns out that, even in the most favourable case (infinitely long rods), the rule of S increasing for increasing water concentration is violated; for example, in the system sodium caprylate–*p*-xylene–water (see Table II), the area S for the rods would be 35 Å^2 in the cubic phase, as compared to 47 Å^2 in the hexagonal phase.

Position *k* of Pm3n allows the choice of two independent parameters and thus involves a large number of possibilities. Nevertheless, none of these is quite satisfactory, especially when the fourth condition is taken into account.

TABLE II

SOME DIMENSIONS OF THE CUBIC PHASE FOR DIFFERENTS SYSTEMS ($T = 22°$)

Φ_l and Φ_{par} are the volume concentrations of the whole lipid and of the paraffin part. $\bar{v}_l$ is the partial specific volume of the lipid. R is the radius of the rods and of the spheres. S is the area per polar group at the interface. In agreement with previous cases (see legends of Tables II and III in ref. 4) the internal volume is assumed to contain the whole soap molecules and the organic solvent in the first system (sodium caprylate–p-xylene) in which the polar moiety is of small size, and only the paraffin chains for the other lipids whose polar moieties are bulky. The volume and the surface of each rod, bevel-shaped to meet with two others at one end, with three others at the other end, have the expressions (see ref. 4); $v = \pi R^2 l\,(1-0.669\,R/l)$: $s = 2\pi Rl\,(1-1.05\,R/l)$, with $l = a/\sqrt{8}$ (length of one rod). Each unit cell contains 24 rods and 2 spheres.

	Sodium caprylate–p-xylene (ref. 6)		*Lysolecithin*		*Lauryl decaethyleneglycol*		*Dodecyltrimethyl-ammonium chloride (ref. 7)*	
	Cubic	*Hexagonal*	*Cubic*	*Hexagonal*	*Cubic*	*Hexagonal*	*Cubic*	*Hexagonal*
$\bar{v}_l$ (cm^3/g)	0.950	0.950	0.937	0.937	1.00	1.00	1.00	
Φ_l	0.46	0.47	0.47	0.49	0.42	0.49	0.43	
Φ_{par}			0.19	0.20	0.14	0.17	0.31	
a (Å)	75	30	148	75	125	59	86	
R_{rods} (Å)	10.7	10.6	13.6	18.0	9.6	12.8	9.9	
S_{rods} (Å^2)	47	47	49	41	71	56	62	
R_{sph} (Å)	15.9		21.3		14.4		14.8	
S_{sph} (Å^2)	49		50		73		63	

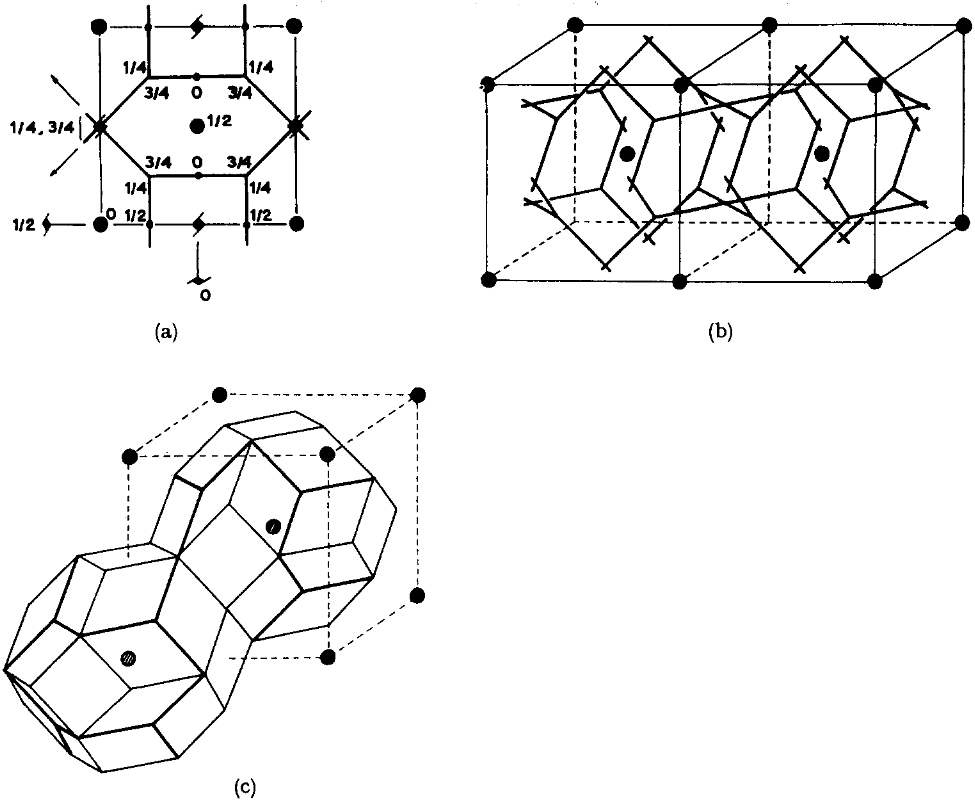
Position j of Pm3n consists of a system of rods lying along 2-fold axes, linked together to form the three-dimensional network discussed below (see Fig. 1). But if the structure contained only this network the area per polar group would be too small as compared to the hexagonal phase (for example, $S = 39$ Å^2 for sodium caprylate, see Table II).

Since no single set of positions appears to be satisfactory, a more complex solution of the problem is sought, involving two sets of special positions. A quick inspection shows that the only pair of positions that meet all the conditions are j and a of Pm3n.

The structure is represented in Fig. 1. It contains a system of rods of finite length, all identical and crystallographically equivalent, joined three by three at one end and four by four at the other (position j), and a body-centred arrangement of spherical elements (or quasi-spherical, since the symmetry of position a is m3). The length of each rod is $a/\sqrt{8}$; each unit cell contains 24 rods and 2 spheres.

The structure can be described (see Fig. 1c) as a body-centred space-filling assembly of identical truncated octahedra. These polyhedra have eight hexagonal and six square faces. The rods sit on the hexagonal faces in positions that do not agree with a body-centred lattice. The cubic cell is thus primitive with two polyhedra per cell. The structure is in fact a chlathrate, formed by polyhedral cages, whose bars are the lipid rods, each cage enclosing one spherical micelle.

Assuming, as discussed above, that the rods and spheres are of Type I (namely that the paraffins are inside), the volume of rods and spheres contained in one unit cell can be determined when the chemical composition, the lattice parameter and the partial specific volumes are known (the partial specific volumes are supposed to



(a)

(b)

(c)

Fig. 1. Representation of the structure. In (a) and (b) the axes of the rods are represented by heavy bars, the limits of the unit cells by thin lines, the centers of the spheres by black circles. The rods are of finite length, and lie along two-fold axes. The rods are joined three by three at one end, four by four at the other. The gaps at the ends of the rods (see text) are omitted. The interior of the rods and spheres is occupied by the paraffin chains, the external volume by the water. The rods and spheres are quite bulky, almost in contact with each other (see text and Table II). (a) Projection on the plane ab, with the position of some of the structure elements. The intersections of four rods are shown by dots. The fractional figures are the z coordinates of the intersections of the rods. (b) Perspective view of two unit cells. Note that the joining rods build up a three-dimensional network. (c) Front view of two cage-like truncated octahedra, each enclosing a spherical micelle. The rods (heavy lines) sit on the hexagonal faces. Note that the packing of the polyhedra is space-filling body-centred: the position of the rods indicates that the unit cell is primitive, with two polyhedra per cell.

be independent of the shape of the structure element). The distribution of the lipids between the rods and the spheres is unknown: the dimensions given in Table II are determined by making the arbitrary assumption that in both elements the area per polar group takes similar values. It can be noted that the rods are of similar radius in the cubic and hexagonal phases, that the radius of the spheres is close to the fully extended length of the paraffin chains, in agreement with what is known for the micellar solutions[9], and that the area per polar group increases as the water content increases. It is clear that the agreement could be improved if the volume of the rods were increased, and the volume of the spheres decreased, thus allowing the area per polar group to be smaller in the rods than in the spheres (see Table II).

As an additional confirmation we have compared the observed and calculated structure factors for the system sodium caprylate–*p*-xylene–water. The electron density distribution is rather odd in this case since the rods and the spheres are bulky, and the contributions of the paraffin cores and of the polar groups must be taken into account (see discussion in ref. 4). The structure factors of the network of rods were calculated for thin rods with a short gap at each end, which takes into account the deformations at the intersections[3]. The length ε' and ε'' of the gaps is that of the rod segment whose volume is equal to the volume lost at the bevel-shaped end. The form factor of the rods is that of a uniform paraffin cylinder, surrounded by a cylindrical shell containing the polar groups of the lipids. The form factor of the spherical micelles is that of a uniform paraffin sphere surrounded by a polar spherical shell. The radii of the paraffin cores and of the polar shells of both the rods and the spheres are determined as discussed previously. The electron density of the polar regions is somewhat arbitrary because the precise space distribution of the counterions is not known[4]; the adjustable parameters K_1 and K_2 of Table I take into account this effect. The formulae and dimensions are given in Table I. The agreement of the observed and calculated intensities is quite satisfactory ($\Sigma|F_0-F_c|/\Sigma|F_0| = 0.19$), although it should be noted that this kind of verification is perhaps less conclusive here than in other cases[1,4], since the number of adjustable parameters is higher (K_1, K_2, α, see Table I).

This phase seems to be the first example in lipids of a structure containing elements of more than one form. Another phase containing two types of structure elements has been reported previously in mitochondria lipids (phase Lγ)[10], but in that case, both elements are lamellar. The presence of two types of structure elements may be postulated as a means of preserving an efficient surface/volume ratio while avoiding too large a separation of the structure elements. Indeed, the maximum distance between rods ($a\sqrt{3}/2$) is found along the 3-fold axes; rods and spheres are intercalated and almost in contact in that direction. The distance between rods along the 4-fold axes ($a/2$) is too short to leave room for a lipid-containing element (see Table II); the gap is filled by water. The structure is less compact than that of the other cubic phase, as the maximum volume concentration, corresponding to structure elements in contact, is 0.51 in this case, 0.874 in the other[4]. Besides, the presence of rods and spheres is a favorable feature for a phase bordered on one side in the phase diagram by the rod-containing hexagonal phase and on the other side by the isotropic solution which in these systems[9] most likely contains spherical micelles.

This structure may be compared to other structures formed by identical rods, all crystallographically equivalent. Several cases have been observed. (a) The rods are indefinitely long: the lattice is two-dimensional hexagonal[1]. (b) The rods are of finite length, and are joined three by three in one plane. Each rod and the four others joined to it may be coplanar: the structure consists then of two-dimensional hexagonal networks (phase R, refs 11 and 12). Otherwise the planes of the rods joining at both ends of a rod may be at right angles: in this case the structure is cubic as space group Ia3d (refs. 1–4). (c) The rods are of finite length, joined four by four. The rods may be coplanar: the lattice is tetragonal (phase T (ref. 12)). The four joining rods may be oriented tetrahedrally: the structure (space group Pn3m) was observed in phosphatidyl ethanolamine[5]. (d) The rods are of finite length, and are joined three by three at one end, four by four at the other: this structure is described in this work. The packing of the rods is less compact than in the other cases, and leaves room for the spherical micelles and water.

As a final comment we wish to stress once more the paradox of a high degree of long-range order accompanied by a liquid-like short-range organization—the structure of most lipid–water phases can indeed be visualized as an ordered distribution of two liquid media, water and hydrocarbons, separated by a continuous surface covered by the polar groups of the lipid molecules. This class of structures appears to be characteristic of lipids, since it is not observed with any other type of chemical compounds. This peculiar distribution of long-range order and short-range disorder, and the remarkable polymorphism of lipids are likely to be related to the biological role of these substances; we have dealt with this question elsewhere[13].

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