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The lyotropic polymorphism of two pharmacologically active molecules†

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The two drugs considered are *amiodarone hydrochloride* (AC), used in heart therapy and *palmitoyl timolol malonate* (PTM), a prodrug of timolol and a medicament for glaucoma. These crystalline compounds are poorly soluble in water at room temperature, but form transparent phases, which are sols or gels, much more concentrated than the critical micellar concentration (CMC), when the crystals are heated in the presence of water to temperatures from 50 to 80°C and cooled to room temperature. These transparent pseudo-solutions were also obtained by dissolution in chloroform, followed by rapid solvent evaporation and addition of water. A lamellar phase was also observed, with its classical textures: myelin forms, oily streaks and polygonal fields. Two transparent and isotropic phases, one more concentrated than the lamellar phase and the other one less concentrated, are presumed to be inverse sponges. The water-rich phase, mainly studied with amiodarone, formed a milky cloud on further dilution and a model has been proposed. From these two examples, some remarks of a geometrical kind are presented on the rich polymorphism of certain water–lipid systems.

1. Introduction

Two amphiphilic molecules of therapeutical interest are considered, their formulae and van der Waals profiles being presented in figure 1:

(1) *Amiodarone hydrochloride* (AC) or Cordarone® is well known for its antiarrhythmic and antianginal properties [1].

(2) *Palmitoyl timolol malonate* (PTM) is a prodrug synthesized by esterification of timolol with palmitic acid [2]. *Timolol* itself is a blocker of adrenalin β-receptors within cell membranes, and is used in collyriums for glaucoma treatments [3]. Timolol decreases the intraocular pressure, after penetration through the cornea, and this can be facilitated by grafting a paraffinic chain onto the molecule, making it soluble within cell membranes. The ester, i.e. the prodrug, is slowly hydrolysed by lipases in different ocular compartments and the active drug can be delivered on a longer delay, closer to target cells and at lower concentrations [4].

These two molecules present complex lyotropic behaviours, showing lamellar phases on polarizing microscopy, with characteristic textures, and also two optically isotropic phases which seem to be sponges of inverse topologies [5, 6]. We present new preparation methods and new observations relating to these phases. We also propose some simple qualitative mechanisms for the origins of micellar shapes and phase transitions.

Instead of considering a unique spontaneous curvature as usual in lyotropic liquid crystals, each monolayer or each bilayer is supposed to have two spontaneous principal curvatures in a given physico-chemical environment [7]; this specifies a local relaxed shape, generally rehandled into one of the three fundamental shapes with constant principal curvatures—spherical, cylindrical or planar.

2. Materials and methods

AC was provided in the form of a white crystalline powder, with a specific purity of 99.4% on HPLC assay, by SANOFI, France [8–10]. Levogyre PTM was synthesized and purified as indicated in [2]. A 50 mg ml^{-1} stock pseudo-solution of AC was prepared by stirring and heating the crystalline powder in pure water, at 70°C

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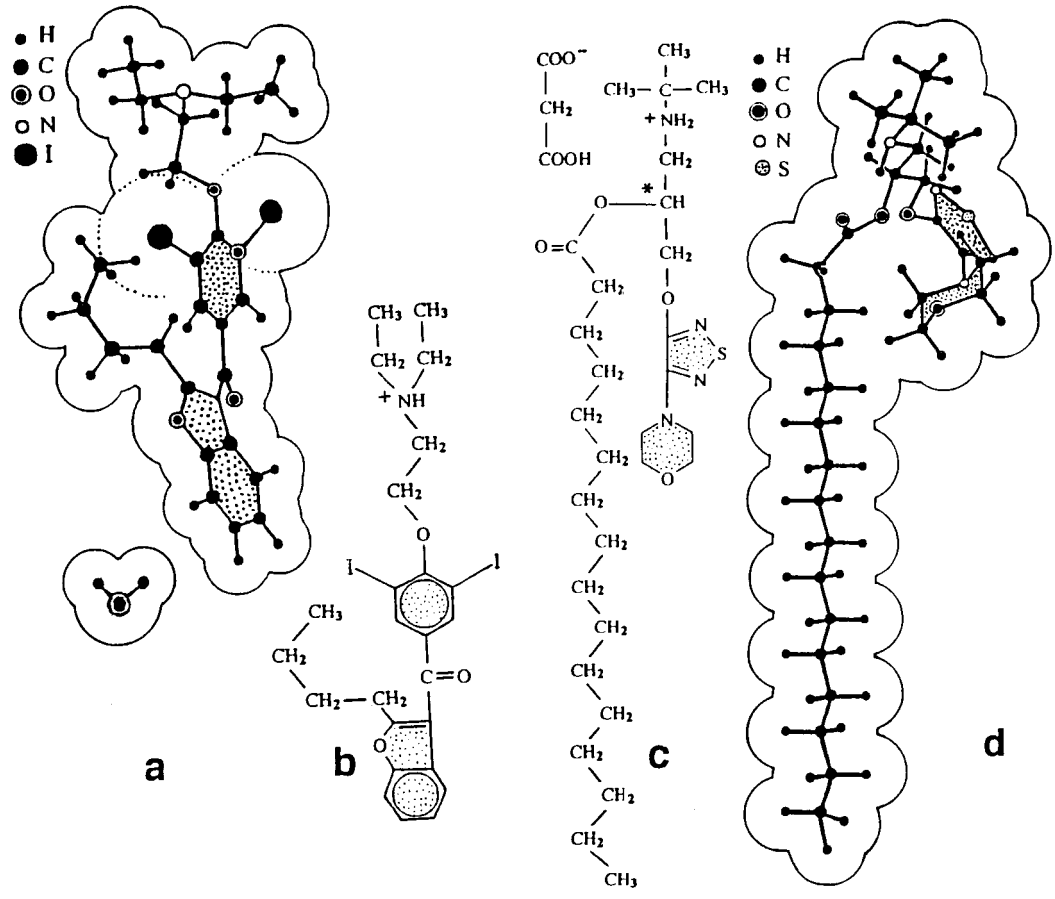

Figure 1. Formulae and van der Waals profiles of amiodarone and palmitoyl timolol malonate. (*a*) Model of amiodarone redrawn after the structure established from X-ray diffraction on crystals [8–10]. To show the scale, the van der Waals contour of a water molecule was added, its longest dimension being 3.9 Å. (*b*) Amiodarone formula: note that we studied the hydrochloride, and that the plane of the di-iodophenyl ring lies normally to that of the heterocycle. (*c*) Formula of palmitoyl timolol malonate and (*d*) its van der Waals profile.

for some minutes, and then cooling to room temperature. Amiodarone was also prepared in 0.10M and 0.15M phosphate buffers (NaH_2PO_4 or Na_2HPO_4), adjusted by addition of drops of H_3PO_4 or NaOH solutions to pH 3.3, 4.4, 6.7.

The PTM was prepared at various concentrations in water, often after heating to 50°C and cooling to room temperature.

These preparations were observed between a slide and coverslip for polarizing microscopy, when birefringent phases appeared due to progressive evaporation of water at the coverslip edge. Heating the preparation at 50°C and cooling to room temperature was necessary in general to observe the lamellar phase of PTM.

Another method was to dissolve AC or PTM in chloroform. A drop of the solution on a glass slide evaporates, forming a dry and transparent deposit; the lamellar phase is then easily observed by adding a water drop and a coverglass. Two polarizing microscopes were used, a Leitz Orthoplan Pol, equipped with phase contrast, and a Zeiss Photomicroscope Axiophot Pol.

Monolayers of AC or PTM were prepared by spreading at the air–water interface, with an Exmire® microsyringe containing a 7 mg ml^{-1} solution of one of these two products in chloroform (Normapur®, Prolabo, France). The aqueous subphase was pure water or was buffered at different pH values in a Langmuir film balance (Lauda FW2, Germany), after cleaning the water surface (927 cm^2) by suction. Isotherms of surface pressure (π) versus surface area (A) were obtained by continuous compression of the spread monolayers at constant velocity (150 cm^2 min^{-1}).

3. Results

3.1. *Transparent sols and gels*

The transparent phases obtained with amiodarone at concentrations from 2 to 50 mg ml^{-1} are not true solutions, but micellar systems or pseudo-solutions, since

the critical micellar concentration (CMC) is close to 0.5 mg ml^{-1} and this is evidenced by the abrupt drop in the electrical conductance to one third of its value at concentrations below the CMC [11]. Surface tension measurements of timolol prodrug (PTM) at pH 6.5 lead also to an estimate of 0.7 mg ml^{-1} for the CMC [5].

3.1.1. *Macroscopic aspects*

The 50 mg ml^{-1} stock preparation of amiodarone was limpid, homogeneous and apparently of weak viscosity, by visual examination. This transparency was maintained in more concentrated preparations, and those ranging from 80 to 105 mg ml^{-1}, also obtained at 70°C, formed transparent gels at room temperature. A white deposit often appeared in these amiodarone gels or sols, after weeks or months at room temperature, and was made of small solid crystals in the form of parallelograms [6]. For 120 mg ml^{-1} and higher concentrations, the gels became turbid at room temperature and 'creaming' occurred in general at the air interface. The transparent gels observed between slide and coverslip showed similar transformations at room temperature, this being due to water evaporation at the coverglass edge, the creamy aspect being that of a lamellar phase, easily recognizable by polarizing microscopy [6]. Finally, the lamellar phase transformed into a transparent and highly viscous product at the end of evaporation. Water added to the transparent gels at room temperature restored the birefringent phase, its fluidity and also the transparent phases, with a milky cloud forming here and there. Dilution of the gels (80–105 mg ml^{-1}) or of the stock preparation (50 mg ml^{-1}) did not alter their transparency for concentrations above 2 mg ml^{-1}. At lower concentrations (0.125–1.5 mg ml^{-1}), the preparations showed a milky opalescence and sedimented slowly, leaving a limpid supernatant liquid.

In the presence of a phosphate buffer, at pH 3.3, the transparent preparation obtained by heating to 70° and cooling to room temperature remained transparent for concentrations below 10 mg ml^{-1}, whereas a milky cloud appeared for concentrations from 10 to 50 mg ml^{-1}, before separation occurred into two transparent phases by slow sedimentation. At pH 4.4, the milky preparation was obtained for higher concentrations of amiodarone, from 25 to 50 mg ml^{-1}, and behaved similarly.

We did not work with large amounts of the timolol prodrug and our observations were mainly qualitative, showing however a polymorphism of phases and textures very similar to that of the water–amiodarone systems. Solid crystals were observed. Several transparent and isotropic phases at concentrations higher than the CMC of 0.7 mg ml^{-1} were obtained directly at room temperature between slide and coverslip. A lamellar phase also was observed with the corresponding textures, sometimes at room temperature, but heating was often necessary to about 50°C, at temperature lower than that required for amiodarone [5].

3.1.2. *Effect of filtration*

The stock pseudo-solution of amiodarone filtered through 0.22 µm Millipore® changed from 50 to 48 mg ml^{-1}. This meant that 2 mg ml^{-1} or 4% of amiodarone remained attached to the filter or elsewhere. The filtration of a 0.5 mg ml^{-1} solution prepared either at room temperature or at 70°C, led in both cases to a 0.25 mg ml^{-1} filtrate, corresponding to a loss of 50% of amiodarone. The loss was 80% with a 0.125 mg ml^{-1} solution and close to 100% with solutions of 0.05 mg ml^{-1} and below. Since there are no chemical reasons for any particular links between amiodarone and Millipore filters, this suggests that all solutions, even very dilute, contain large amiodarone aggregates, which easily attach to filters, and that they cannot be considered as true solutions. We therefore prefer to use the term *pseudo-solutions*.

3.2. *Microscopic observations*

3.2.1. *Saturated solutions of amiodarone*

We tried to prepare amiodarone solutions at room temperature and to observe them in the microscope. Since this compound is poorly soluble in water, possibly at a maximum of 0.7 mg ml^{-1} [12], the crystalline powder did not seem to dissolve and the crystals remained intact, but apparently since there is dissolution, this is weak but real. These crystals were mounted between a slide and coverslip in the presence of water saturated or supersaturated with amiodarone and examined by phase contrast microscopy, with the immersion objective; we observed here and there some rare myelin figures, very tiny, and forming narrow and branched tubes of lamellar material, the longest dimension lying between 1 and 3 µm.

3.2.2. *Sols and gels less concentrated than the lamellar phase*

Most of the amiodarone samples that we mounted between slide and coverslip were transparent gels (80 to 105 mg ml^{-1}) and sols, like the stock pseudo-solution (50 mg ml^{-1}) or those diluted in pure water, above 2 mg ml^{-1}. These gels and sols were perfectly isotropic without any differentiated structures at this resolution, in polarizing or in phase contrast microscopy. We observed in certain preparations a more refringent phase, but not birefringent, with rectangular contours well differentiated in phase contrast microscopy, suggesting a transition to a cubic lyotropic structure. However, these aspects could be the result of NaCl crystallization, since amiodarone

is a hydrochloride and glass is made of alkaline silicates. Direct addition of NaCl to the amiodarone preparation led to similar structures and many more developed.

Shear due to local pressure of a needle on the coverslip or to a displacement of the latter often led to a brief flash of chromatic polarization, indicating flow birefringence in the viscous preparations of isotropic amiodarone [6].

Water evaporation at the coverslip edge resulted in the formation of birefringent stripes after a delay depending on concentration. These stripes corresponded to textures which are generally classical in lamellar phases of lyotropic systems.

3.2.3. *The milky opalescence*

When transparent preparations such as the stock pseudo-solution of amiodarone and gels were diluted below 2 mg ml^{-1}, they gave a milky opalescence, mainly in the range from 1.5 to 1.25 mg ml^{-1}, which appeared in the microscope to be an emulsion of two isotropic phases. This milky opalescence was also observed in the dispersion of PTM at low concentrations, but we did not study it in detail as we did for amiodarone.

A drop of the amiodarone pseudo-solution (50 mg ml^{-1}) was deposited onto a preparation slide and then immersed and surrounded in a drop of pure water, and finally sandwiched by a coverglass. The central drop was laterally diluted with a concentration gradient which was radially distributed. The milky opalescence appeared along a regular ring, whose diameter progressively increased, see figure 2(*a*). This allowed one to observe the small droplets responsible for the turbidity with their various diameters around 1 µm, the largest ones reaching 5 µm. However, these drops also assembled into more extended systems, particularly after settling and adhering to the slide, due to a higher density of their more concentrated phases, amiodarone being a heavy molecule with its two iodines [6]. The interface between the two phases appeared very sharp for droplets, but surprisingly this interface seemed to be absent between the two widely extended phases, that coming from the pure water and that from the stock pseudo-solution. Our interpretation is sketched in figure 2(*b*) and supposes that this interface joins the slide and coverslip obliquely. This obliquity is also due to the higher density of the isotropic phase with a higher amiodarone concentration. The interface is close to being horizontal and extends within the turbid zone, which could explain its lack of contrast.

3.2.4. *The lamellar phase*

When the limpid stock preparation of amiodarone was made turbid by dilution in water, and then submitted to slow evaporation between slide and coverslip, a long series of transformations was observed, with the coalescence of heavy phase droplets, and birefringent stripes appearing at the interface of the two isotropic phases. Starting from a limpid preparation, with concentrations ranging from 2 to 50 mg ml^{-1}, the slow evaporation also led to the differentiation of birefringent stripes, obtained more rapidly in the case of a 100 mg ml^{-1} gel.

A large series of textures appeared in the course of dehydration of the amiodarone lamellar phase. They are well known in most lyotropic lamellar phases, namely in phospholipids such as natural lecithin. The nucleation of these smectic systems generally occurred at the periphery of the coverslip. The loss of water had two opposite effects: either a decrease of the preparation thickness, or an increase when air bubbles formed. The first process resulted in horizontal alignments of bilayers, whereas the second one led to flow, mainly horizontally in the preparation plane, bilayers being tilted here and there. The resulting textures were myelin forms, oily streaks and polygonal fields, interpreted as the successive steps in the dehydration process [6]. Results were similar for PTM, but the polygonal fields were very rare, whereas a cellular texture corresponding to a particular arrangement of oily streaks was often found after heating at 50°C [5].

As indicated above, these textures were obtained much more rapidly and in a very reproducible way, simply by adding a drop of water onto the dry deposit

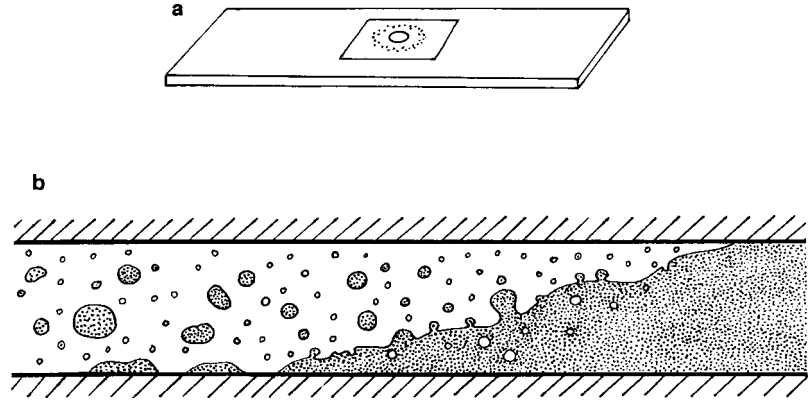

Figure 2. Experiment on the milky ring between slide and coverslip (*a*) and its interpretation (*b*).

of amiodarone or timolol prodrug, obtained from the evaporation of a small drop of a chloroform solution on a glass slide. The first effect was the production of Maltese crosses between crossed polarizers, and their aspect is shown for amiodarone in figure 3(*a*), when a first order retardation plate λ was added. The positions of the orange and blue sectors indicated that the ellipsoid of indices appeared in section as an ellipse with a radial short axis and a tangential orientation of the long axis. These Maltese crosses transformed rapidly into finger-like structures, which were cylindrical myelin forms, elongating horizontally between slide and coverslip, figure 3(*b*). More precise observations showed that the first Maltese crosses corresponded to the nucleation of domes (with concavities directed downwards), becoming hemispherical and then cylindrical, with a rounded extremity. These myelin forms seen horizontally showed more or less regular constrictions, as they appear in the Plateau problem to minimize the surface energy of a liquid vein [13].

Water evaporating at the coverglass periphery and the loss of preparation thickness modified the aspect of the myelin fingers in a first step, with bilayers arranging horizontally for some of them, figure 3(*c*). In a second step, the myelin cylinders became sinuous and the horizontal layers corrugated. In a third step, the non-horizontal layers formed oily streaks, figure 4(*a*), whereas the horizontal layers, already corrugated, transformed into polygonal fields, figure 4(*b*).

The milky cloud of amiodarone obtained in the presence of a phosphate buffer at pH 3.3 (or 4.4), for concentrations from 10 to 50 mg ml^{-1}, (or 25 to 50 mg ml^{-1}), showed in the microscope an emulsion of two isotropic phases, forming long threads extending along the stream lines produced by the coverslip, figure 4(*c*). At rest, one of the two phases appeared in the form of refringent 'tears' attached to the slide by narrow pedunculi, figure 4(*d*). A 50 mg ml^{-1} pseudo-solution in phosphate buffer, at pH 4.3, also showed these tears, between slide and coverslip, and then the addition of a drop of buffer at pH 6.7 immediately produced the nucleation of the lamellar phase, figure 3(*d*). A similar effect was obtained by the addition of a drop of NaCl solution containing 9 mg ml^{-1}.

3.2.5. *The transparent concentrated phase*

At the end of the evaporation of a pseudo-solution of amiodarone in pure water, the lamellar phase becomes transparent and isotropic, with a high viscosity. There is a sharp interface separating this phase from the birefringent lamellar systems [6]. In some preparations, small crystals, devoid of birefringence and presenting rectangular profiles were also observed within these concentrated isotropic phases of amiodarone, as in certain gels less concentrated than the lamellar phase, but the presence of NaCl crystals is plausible, as suggested above.

Brilliant rodlets (chromatic polarization), with a texture very close to that known in columnar hexagonal phases, also appeared in the highly viscous, isotropic and transparent phase of amiodarone, but only when the immersion oil penetrated between slide and coverslip, by pure mishandling; this probably corresponded to an inverse hexagonal liquid crystal. A similar texture was observed at the end of evaporation of the timolol prodrug preparations, but here in the absence of immersion oil.

3.3. *Comparison of the two drugs for their lyotropic polymorphism*

In the absence of water, the crystalline PTM showed a melting point close to 50°C. These crystals were partially soluble in water, and this was facilitated by heating to 50°C. On the contrary, in the absence of water, the amiodarone crystals were modified chemically before melting near 150°C; a brown colour was produced, but recrystallization did not occur on cooling, even in presence of amiodarone crystals introduced as seeds. In the presence of water, amiodarone crystals dissolve at 66°C, giving a clear pseudo-solution at room temperature.

The lamellar phase of timolol prodrug is easily obtained after a brief heating of preparations in water at 50°C, for concentrations ranging from 12 to 80 mg ml^{-1}, whereas for amiodarone heating to 70°C is necessary before cooling, the lamellar phase being observed in pure water at room temperature for concentrations ranging from 120 to 400 mg ml^{-1}.

The most striking difference between the two drugs was the presence, in the timolol prodrug, of an interface separating the two isotropic phases, one less concentrated and one more concentrated than the lamellar phase; such interfaces were absent in amiodarone preparations in pure water. In the timolol prodrug preparations, lumps of lamellar phase attach here and there to the clear cut interface separating these two isotropic phases, and this situation is encountered in a very large range of concentrations, from 10 to 90 mg ml^{-1}. On the contrary, in amiodarone preparations in pure water, the lamellar phase separates these two isotropic phases.

Finally, let us underline a strong similarity between these two molecules: monomolecular films of AC and PTM were studied in the Langmuir film balance and the surface pressure measured versus the molecular area is shown in figure 5. By pure coincidence, the minimal area per molecule was 43 Å^2 for both molecules [5, 14].

4. Discussion

4.1. *Anterior observations on tensioactive properties*

Amiodarone hydrochloride is a well known molecule, figure 1(*a*,*b*), whose geometry was deduced from the X-ray diffraction produced by crystals grown in ethanolic

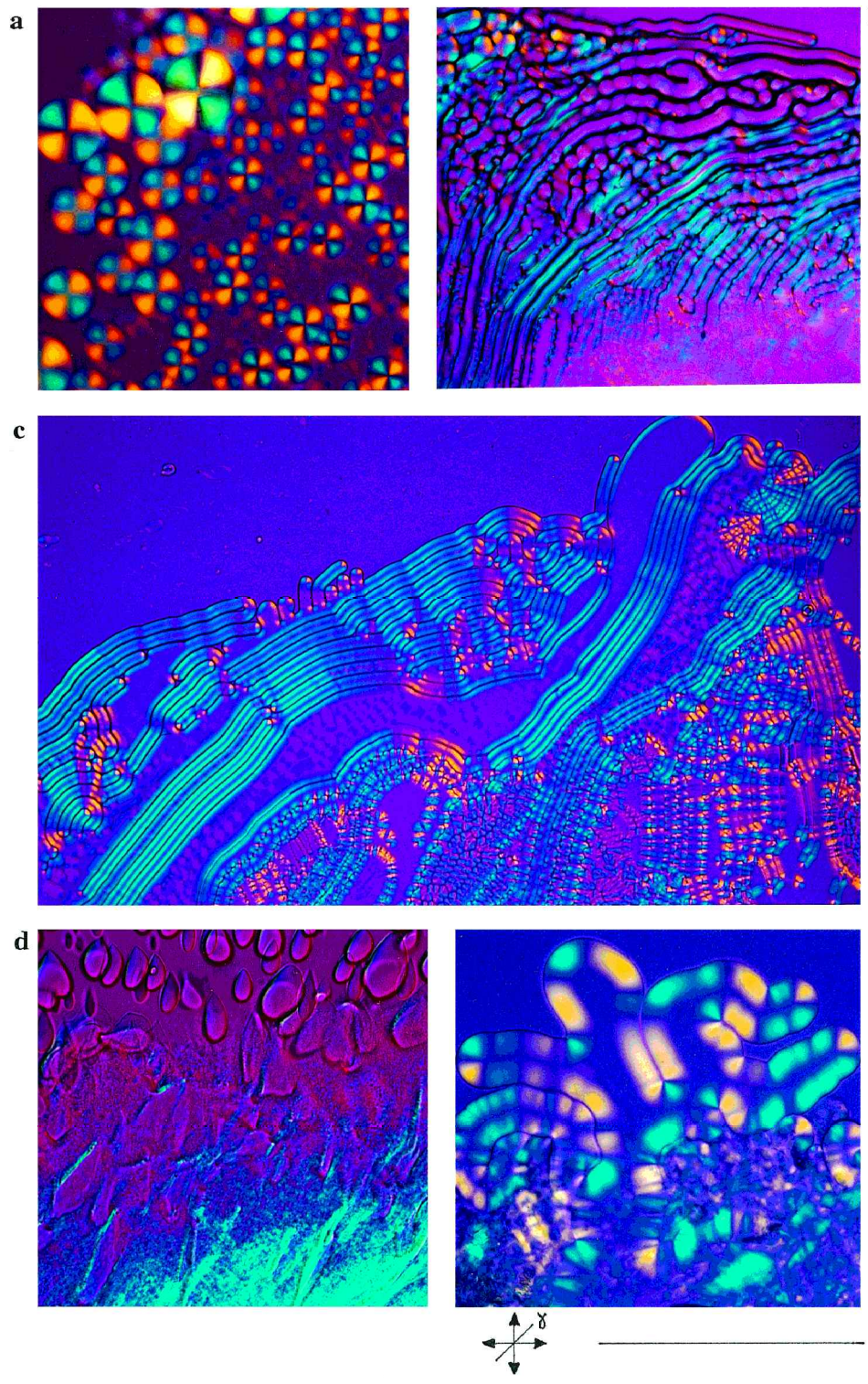

Figure 3. Lamellar phases in water–amiodarone (*a–d*) and palmitoyl timolol systems (*e*), with crossed polarizers and first order retardation plate λ at 45°; introduction of a Wollaston prism for the Nomarski interferential contrast in (*d*). (*a*) Myelin figures appear some seconds after the addition of a water drop onto a dry film of amiodarone, obtained from the evaporation of a chloroform solution. (*b*) Amiodarone: finger-like myelin figures develop rapidly, but the birefringence is variable and close to zero in some of them. (*c*) Myelinic amiodarone is very fluid and water evaporation leads to the first steps in the differentiation of textures such as polygonal fields and oily streaks (see figure 4); a flattened myelin finger crosses the micrograph, diagonally from bottom left with 'corrugated' layers, to top right with horizontal layers. (*d*) Amiodarone 'tears' in a 0.1M phosphate buffer, at pH 3.3, and transition to a smectic phase (blue area) by the addition of a drop at pH 6.7. (*e*) Myelin figures in a 20% dispersion of the malonate of palmitoyl timolol in water, after a brief heating to 50°C. Bar = 100 μm for (*a*) and (*e*), and 200 μm for (*b*, *c*, *d*).

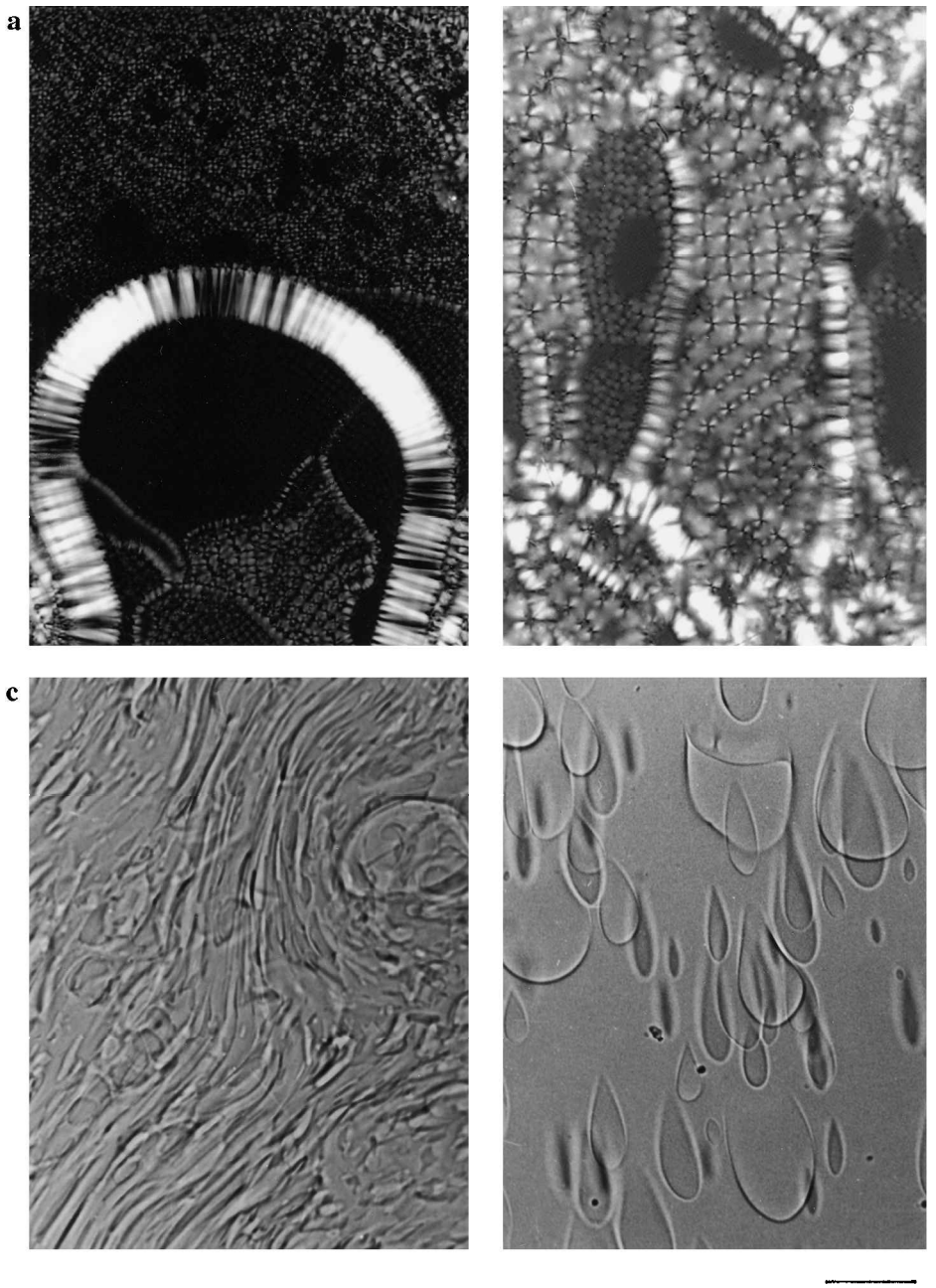

Figure 4. (*a*, *b*) Amiodarone lamellar phase in presence of pure water, observed between crossed polarizers. (*c*, *d*) Coexistence of two isotropic phases in a 50 mg ml^{-1} amiodarone preparation in a phosphate buffer at pH 3.3, after heating to 70°C, observed between parallel polarizers at ordinary temperature. (*a*) An 'oily streak' appears as a bright band separating two dark zones with horizontal bilayers; the oily streak with its characteristic cross-striation corresponds to a set of vertical or strongly oblique bilayers. (*b*) Polygonal fields extending between oily streaks. (*c*) The milky emulsion obtained by cooling a drop of the amiodarone preparation between slide and coverslip. (*d*) The same preparation some minutes later forming a set of 'tears'. Bar = 20 µm.

solutions [8–10]. The timolol prodrug was synthesized from levogyre timolol malonate esterified by a palmitoyl chain, and its conformation obtained from NMR studies was published elsewhere and indicated a stiff paraffinic chain at ordinary temperature [2]. The resulting product, after formation of the salt with malonic acid, is represented in figure 1 (*c*, *d*). Previous physico-chemical studies on amiodarone underlined its tensioactive properties [6, 10–12], and the presence of a lamellar phase, showing a birefringence inversion in the course of water evaporation [6]. This indicates that swollen lamellar phases are present. The ellipsoid of indices

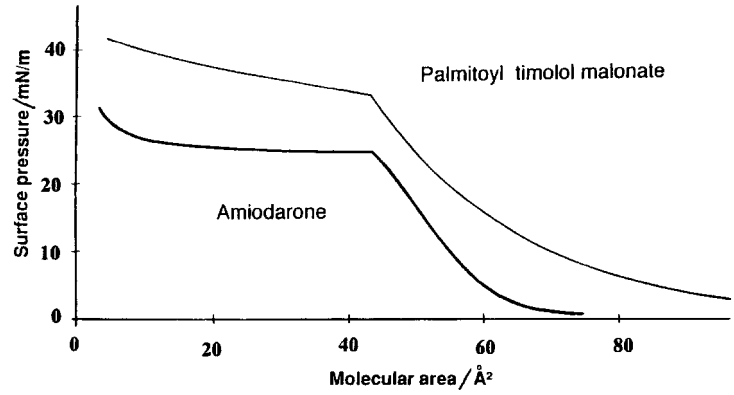

Figure 5. Compression isotherms of palmitoyl timolol malonate and of amiodarone monolayers, on a buffered subphase at pH 6.5.

presents a rotational symmetry about an axis normal to the lamellae. At high concentrations, this ellipsoid is elongated, whereas an increase in the water fraction makes it first spherical and finally flattened. This negative uniaxial birefringence is due to the addition of a form birefringence term corresponding to the presence of intercalated water lamellae, and which is therefore negative [6].

In the isotropic and transparent phase of amiodarone, at 46 mg ml^{-1}, less concentrated than the lamellar phase, an ultracentrifuge study showed a strong heterogeneity of the micelle weights, with a peak slightly over 100 000, representing the half population of aggregates. This weight corresponds to micelles of about 150 molecules [11]. For a molecular length of 17 Å and an external area of at least 43 Å^2, this gives elongated micelles, some being spherical and others cylindrical. NMR studies have attributed the solubility above 60°C to the breakdown of micelles [15], but this did not mean that the stable and transparent preparations obtained by cooling to room temperature were true solutions, since these clear liquids, with a high amiodarone content (50 mg ml^{-1}), cease to be transparent when diluted to concentrations close to the CMC; this is also the case for the timolol prodrug. The low CMC values of 0.5 mg ml^{-1} for amiodarone and 0.7 mg ml^{-1} for PTM, like the important loss of amiodarone by filtration at various concentrations, also indicate that these preparations differ from true solutions.

This was confirmed for amiodarone by light scattering and photon correlation results [6], consistent with microscopic observations of the milky aspects. Hydrodynamic dimensions ranged from 0.1 to 0.5 µm in amiodarone sols at 4 mg ml^{-1} [6]. Neutron scattering showed the presence of cylindrical micelles of 40 Å in diameter, in the 50 mg ml^{-1} stock pseudo-solution prepared in D_2O [16]. This suggests that the transparent sols contain cylindrical micelles separated by 0.1 µm and more, according to concentration, possibly with branchings separated by similar distances.

4.2. *Lyotropic polymorphism*

The hypothesis of amiodarone aggregates taking the form of cylindrical micelles in isotropic sols and gels less concentrated than the lamellar phase is strengthened by the fact that this lamellar phase intercalates in the phase diagram between two transparent and isotropic phases [17, 18]. This situation is encountered in many lyotropic systems, the two isotropic phases corresponding to inverse cubic structures, whose description is very advanced [19, 20]. These cubic phases are made of cylindrical micelles associating by their extremities, three by three, four by four, or six by six, forming two intertwined labyrinths separated by a toroidal layer of water. The inverse structures correspond to cubic phases formed by a toroidal bilayer separating two water compartments, made themselves of cylindrical segments associated by their extremities into similar labyrinths. These bicontinuous structures are often replaced by systems of equivalent topologies, which are either random networks of cylindrical micelles or multiconnected toroidal bilayers, without the discrete order of certain cubic symmetries [21–24].

4.2.1. *The lamellar phase*

The water–amiodarone birefringent phase obtained between the two isotropic gels was lamellar, with well characterized textures. The possibility of hexagonal or any columnar structures was ruled out by the presence of myelinic systems, oily streaks and polygonal fields, three textures absent from columnar phases, since they show considerable amounts of splay, a curvature forbidden in columnar phases. The birefringence inversion of the lamellar phase indicated large variations of hydration within these swollen smectics, due to steric or electrostatic repulsions, or both.

4.2.2. *The isotropic phases*

The hypothesis of two inverse sponges is plausible, since these isotropic phases did not show polyhedral contours, despite some exceptions considered above due

probably to the presence of small amounts of NaCl. Further studies, X-ray diffraction mainly, will indicate the presence or not of cubic order. The passage from a sol to a gel in amiodarone pseudo-solutions less concentrated than the lamellar phase could be due to the transition to a cubic phase, but this must be verified. The following models will concern the areas compared and occupied by the hydrophilic and lipophilic extremities of the amphiphilic molecules, and hold for cubic systems, as well as for sponges of similar topology.

4.2.3. *Tentative phase diagram*

Two inverse sponges, made either of branching cylindrical micelles or of a toroidal bilayer, are drawn in figure 6 to show the supposed topological nature of the isotropic phases, without considering the presence or not of a discrete cubic order. These models are presented in the frame of a 'tentative' phase diagram of water–amiodarone systems, with two axes: concentration and temperature. Six curves separate different states: the diluted true solution, the free micelles, the milky emulsion, the isotropic sol, the isotropic gel, the lamellar phase and the concentrated isotropic gel. Curves I and II are redrawn after results due to Ravin *et al.* [11]. The vertical parts of curves I and II indicate a nearly constant CMC in the range from 20 to 50°C [11]. The positions of curves IV, V and VI are only qualitative, since they were deduced from heterogeneous preparations, with strong concentration gradients and variable thickness. This diagram shows the relative positions of phases at room temperature (near the *C* axis). No particular domain was assigned to the birefringent crystals, similar to those produced from ethanolic solutions, which grew so slowly within the isotropic gel that this gel seemed to be completely stable [6]. Most phases considered in this diagram are therefore metastable, but this corresponds to a long term metastability, even at room temperature. A similar type of metastability was encountered with the timolol prodrug.

The water–amiodarone system is quite unusual, particularly in relation to defining a Krafft point, since the melting temperature of the solid crystals in water is 66°C, and therefore much higher than the temperature of the observed lyotropic phases, the lamellar phase for instance, normally studied at room temperature. The way to reach most of the lyotropic phases, when we start from the solid crystal, is to heat these crystals in water to a temperature supposed to correspond to the

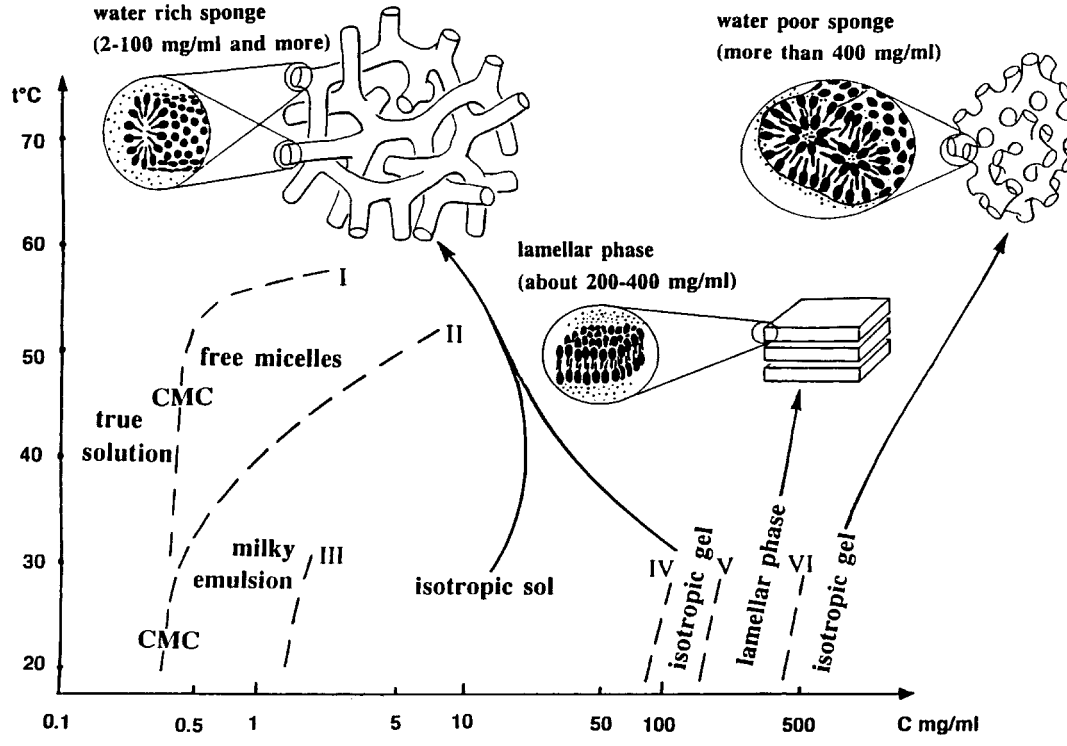

Figure 6. Tentative phase diagram of amiodarone preparations in pure water, presented as functions of amiodarone concentration and temperature. We really explored the part of the diagram of ordinary temperature, the curves III, IV, V and VI, whereas at higher temperatures the curves I and II are redrawn from the quantitative results due to Ravin *et al.* [11]. We have represented the lamellar phase as a swollen smectic, intercalating between two inverse sponges, the water-poor sponge made of a toroidal bilayer separating two water compartments (a bicontinuous system), whereas the water-rich sponge is supposed to be made of a branching system of cylindrical micelles.

breakdown of micelles [15] and to cool to room temperature. This resembles the monotropism usually described in thermotropic mesophases [25].

4.3. *Proposed models*

4.3.1. *Modulated interpenetration of lipophilic moieties*

We show in figure 7 how the van der Waals shapes of the two compounds considered in this study are 'adapted' to form cylindrical micelles or bilayers, and how they pass from the first form to the second, simply by interdigitating their lipidic chains. This model facilitates the interpretation of the polymorphism, either structural or textural.

4.3.2. *Progressive divergence of molecules within monolayers*

The passage from a water-rich sponge to an amiodarone-rich one is depicted in figure 8, and illustrates a point of view defended in ref. [7], *the mean curvature of monolayers varying as a quasi-monotonous function of water concentration*. We start from the top, with a positive maximum of mean curvature, at the level of cylindrical micelles. This mean curvature is lowered at the triple junctions of micelles and along bilayers, where it is close to zero. However local curvatures of 'hat shapes' maintain the mean curvature of monolayers slightly above zero, at least globally, since in that case the area of the lipophilic midlayer shows an area less extended than the mean area of the two corresponding hydrophilic levels. Planar bilayers just below in the model of figure 8, and their local deformations with a cylindrical symmetry as in edge dislocations, are neither 'hat'- nor 'saddle'-shaped. On the contrary, the formation of toroidal junctions, which are strongly saddle-shaped, introduces a globally negative mean curvature of the monolayers, since each hydrophilic head occupies an area less than the area of the corresponding lipophilic end; this is strongly reinforced in the amiodarone-rich sponge, at the bottom of the diagram.

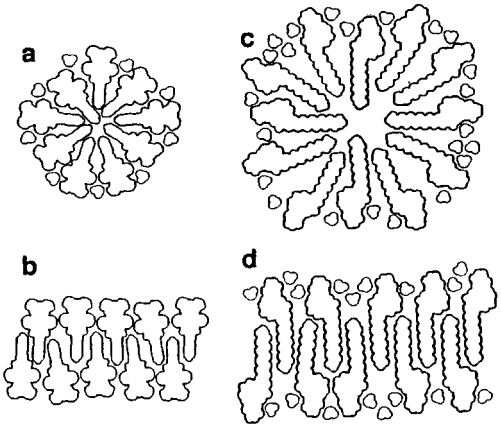

Figure 7. Supposed arrangements of amiodarone (a, b) and palmitoyl timolol molecules (c, d), in cylindrical micelles (a, c) or in bilayers (b, d), with more or less numerous water molecules intercalated between the polar extremities.

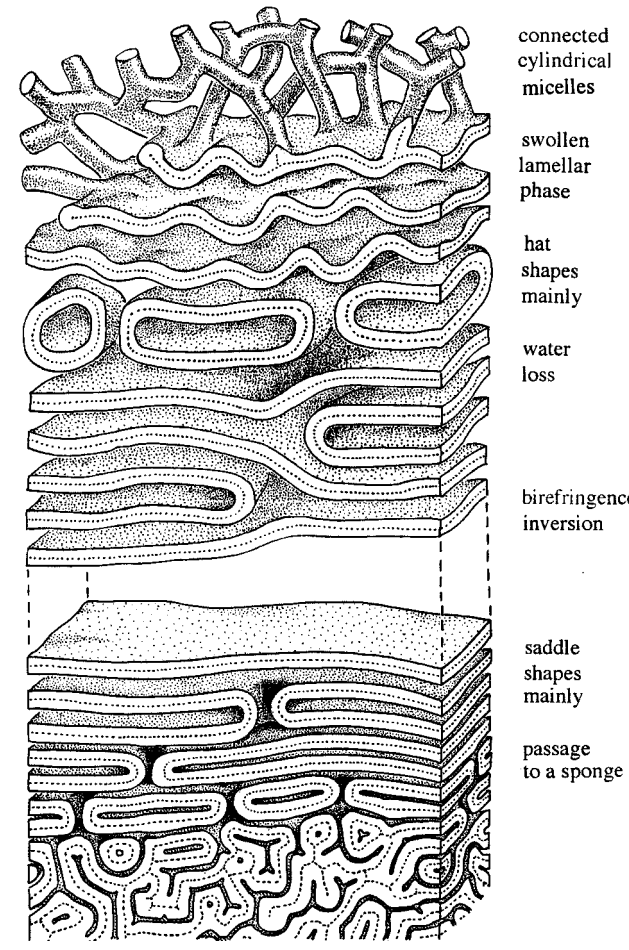

Figure 8. Supposed steps from the water-rich sponge to the water-poor sponge in amiodarone lyophilic systems.

For the timolol prodrug dispersions, the two isotropic phases often coexist, separated by a clear cut interface, the smectic phase being absent or lying attached to this interface. This indicates that the two preferred states are the cylindrical micelles and a bilayer spontaneously toroidal due to an area of the lipophilic level slightly larger than the mean area of the corresponding hydrophilic levels, as argued in [7]. Raising the temperature to 50°C increases the hydrophilic area per molecule and the lamellar phase which appears can be maintained at room temperature, but in a constrained state.

4.3.3. *The origin of opalescence at low amiodarone concentrations*

The micellar network of amiodarone pseudo-solutions extends progressively with dilution, but this process is limited, and holes appear here and there; finally the

network separates into shreds of one or several micrometers in diameter as represented in figure 9, and this produces a milky cloud. The sharpness of the droplet interface is supported by phase contrast observations, often indicating the development of lamellar structures at the interface of sedimented droplets attached to the lower glass of the preparation.

Note also that amiodarone hydrochloride is the salt of a weak base and a strong acid. The pH 3.5 of the 50 mg ml^{-1} pseudo-solution is progressively increased to 4.0 by dilution to the 1.25 mg ml^{-1} concentration, when the milky cloud appears, and some quaternary ammonium salt can be supposed to transform into the amine, the pK_a being estimated between 5.6 and 8.7 [26–28]. This process can slightly decrease the repulsive force between molecules, and bilayer reformation is plausible.

4.3.4. *Textural polymorphism*

The transformations in the lamellar phase show a progressive passage from mainly hat-shaped bilayers in the nucleation of myelin figures, to cylindrical or planar bilayers in elongated and flattened myelinic systems; saddle-shapes are mainly represented in polygonal fields and in cross-striated oily streaks. Within these textures, the hat shapes seem to be localized exclusively along focal curves, representing sites with a decreased degree of interdigitation of the lipophilic parts of molecules. A similar evolution of curvatures was also described by Boltenhagen *et al.* in a swollen lamellar phase of a surfactant in the vicinity of a micellar phase, or in the vicinity of a sponge made of toroidal bilayers [29, 30]; this illustrates a general principle of the bilayer morphology discussed in [7].

4.4. *Limits of the structural investigation*

Small angle X-ray diffraction and cryofracture are among the techniques appropriate for obtaining structural information on lyotropic phases, but the supposed water-rich sponge presents a difficult situation for X-rays, due to the random structure. Cryofracture replica do not suffice in general to recognize random sections of such dispersed cylindrical micelles, with a diameter less than 40 Å. Near field microscopy, AFM for instance, is inappropriate to study phases such as sponges, since evaporation leads rapidly to phase transitions. The recently developed methods of examination of vitrified phases, avoiding water crystallization, could be very useful.

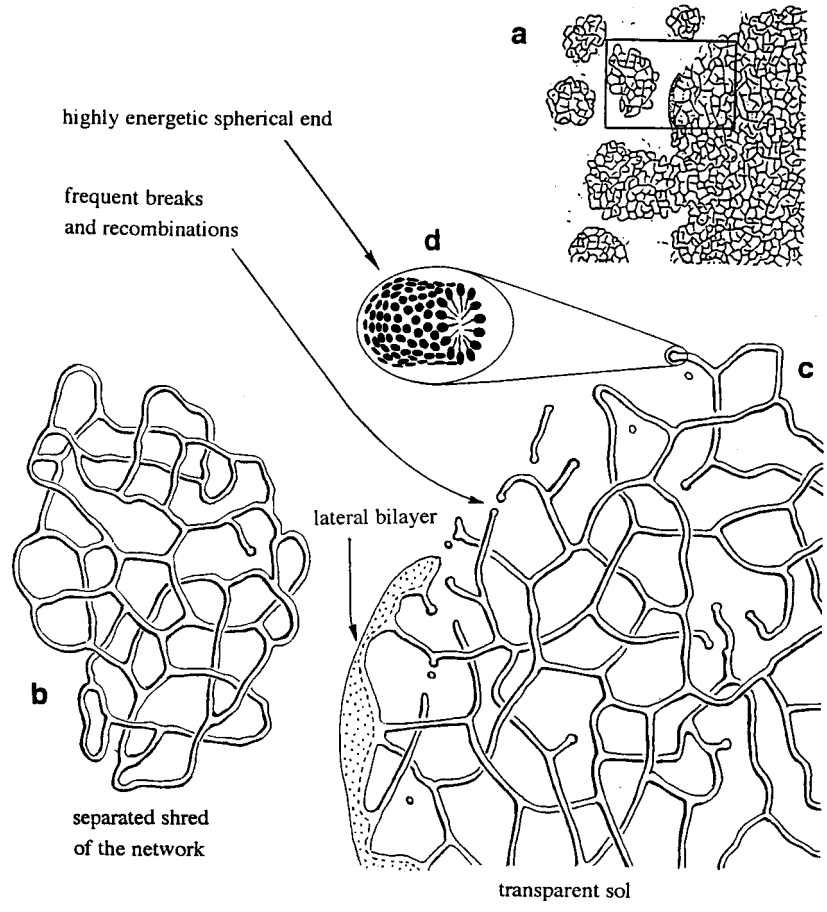

Figure 9. Supposed origin of the milky cloud by dilution of the transparent amiodarone sols. (*a*) The reticulum of cylindrical micelles disjoins into separated shreds and a detail of the rectangle is enlarged in (*b*) and (*c*). (*d*) Molecular organization of a micellar end.

They were applied by Lin [31] to aqueous dispersions of alkylamine oxide and alkyl ethoxylate sulphate mixtures at high pH or salt concentrations. His pictures are very close to the model of figure 9, but free ends of cylindrical micelles seem to be absent.

4.5. *Liquid crystalline polymorphism of other pharmacological molecules*

Studies on liquid crystals are not rare in pharmaceutical periodicals, since many amphiphilic compounds are used in the field of galenic preparations, and more recently in research on liposomes. Much fewer studies concern pharmacologically active agents. One example however is that of *fenoprofen*, a non-steroidal anti-inflammatory substance, presenting a smectic phase, easily prepared in the form of multilamellar vesicles or pharmacosomes, which need nevertheless to be supplemented with some phospholipids to be stable [32].

The interest in amiodarone in the field of liquid crystals was originally pointed out by Chatelain *et al.* [33] in a study of multilamellar and liquid crystalline vesicles, prepared *in vitro* with neutral phospholipids, in the presence of amiodarone or cholesterol. From fluorescence polarization experiments, they showed that amiodarone increases the lipid order parameter, as cholesterol can do. It must be recalled that amiodarone is responsible for adverse events, mainly due to high doses, and the tissues concerned are those in which multilamellar lysosomal bodies are often observed [34]. These cell organelles are liquid crystalline and recall those studied by Chatelain *et al.* [33]. Since amiodarone forms smectic liquids, some of these multilamellar systems could be mainly amiodarone and not membranes with a certain amount of amiodarone.

5. Conclusions

The liquid crystalline polymorphism of these two pharmacological drugs leads us to ask new questions about these molecules when they are considered in their biomedical context. The phase diagrams should be studied in detail, not only in presence of water, but also when ions are added, in particular those found in lymph, blood plasma, and within the ocular tissues in the case of PTM. These molecules dissolve rapidly within cell membranes and can accumulate in certain tissues; for amiodarone the heart is the first concerned, but also the lungs, thyroid, liver, eyes, skin, nervous system, etc., with development of multilamellar structures visible in cell ultrastructures [1]. These multilamellar systems could be mainly amiodarone, instead of membranes with a certain amount of amiodarone, as generally supposed. This means that phase diagrams also should be investigated in the presence of membrane phospholipids and that more cytological studies are required.

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